NATIONAL HEART, LUNG, AND BLOOD INSTITUTE
DIVISION OF INTRAMURAL RESEARCH

THIRD ANNUAL
FELLOWS RETREAT

MAY 12-13, 2005

Harbourtowne Resort and Conference Center
St. Michaels, Maryland.

Fellows Advisory Committee

Dr. Michael Adams, Laboratory of Molecular Immunology
Dr. Martin Crook, Cardiovascular Branch
Dr. Luca Di Noto, Laboratory of Biochemistry
Dr. Heather Jones, Pulmonary-Critical Care Medicine Branch
Dr. Mihaly Kovacs, Laboratory of Molecular Physiology
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Dr. Emily Rothstein, Laboratory of Cardiac Energetics
Dr. Phillip Scheinberg, Hematology Branch
Dr. Yong Tang, NHLBI FELCOM Representative
Dr. Andrew Wragg, Cardiovascular Branch

With Special Help from
NHLBI Office of Science and Technology
DIR Office of Education

Thursday, May 12th

8:00 - 9:30 am	**Arrival & Continental Breakfast** - Prospect Bay Ballroom
9:30 - 9:45	**Introduction & Welcome** - Prospect Bay Ballroom Dr. Heather Jones, *PCCMB* Dr. Herbert Geller, *Director, Office of Education, DIR* Dr. Robert S. Balaban, *Scientific Director, DIR, NHLBI*
9:45 - 11:00	**Scientific Speaker** Chair: Dr. Ryan Morris, *LKEM* Richard P. Lifton, M.D., Ph.D., *Chairman, Department of Genetics, Sterling Professor of Genetics, Professor of Medicine (Nephrology) & Molecular Biophysics & Biochemistry, Investigator, Howard Hughes Medical Institute, Yale University*
11:00 - 12:30	**Research Highlights by NHLBI Fellows** (I) Chair: Dr. Lina Li, *LBG*
11:00	Dr. Robert Fenton, *LKEM*, “Role of Urea in Renal Function: Studies in Urea Transporter Knockout Mice”
11:30	Dr. Mihály Kovács, *LMP*, “Load-dependent Kinetics of Non-muscle Myosin II”
12:00	Dr. Ivan Rosas, *PCCMB*, “Sub-Clinical Interstitial Lung Disease in Familial Idiopathic Pulmonary Fibrosis”
12:30 - 1:30	Lunch
1:30 - 3:00	**Research Highlights by NHLBI Fellows** (II) Chair: Dr. Kirsten Remmert, LCB
1:30	Dr. Ruth Seggewiss, *HB,* “In vitro culture during retroviral transduction improves thymic repopulation and output after TBI and autologous PBPC transplantation in rhesus macaques”
2:00	Dr. Felipe Lisboa, “Glucocorticoids Suppress Mast Cell Activation by Different but Complementary Mechanisms”
2:20	Dr. Wilmar Patino, *CB*, “FOS as a Novel Marker of Atherosclerosis”
2:40	Dr. Hang Wang, *DNG*, “Sulfation as a signal for axonal guidance by proteoglycans”
3:00 - 5:00	**Free Time & Check-In**
5:00 - 7:30	**Fellows Poster Session** - Fairway Room NHLBI Cores and Offices
6:00	**Group Photo** - Bayside Deck

7:30 - 8:30 **Dinner**

8:30 - 9:00 **NHLBI Directors Talk** - Prospect Bay Ballroom
Chair: Dr. Emily Rothstein, LCE
Elizabeth Nabel, M.D.
"Prescription for a Successful Postdoctoral Fellowship: Obligations and Responsibilities of Fellows and Mentors"

Awards Presentation - Prospect Bay Ballroom
Presenters: Drs. Marina Lee, LDB and Andrew Wragg, CB
Fellows Award for Research Mentoring
Fellows Retreat Research Award

9:00 - 12:00 **Social Time**
Dance to DJ Music by Robert Walls

Friday May 13th

8:00 - 9:15 **Breakfast**

9:30 - 10:45 **Keynote Speaker** - Prospect Bay Ballroom
Chair: Dr. Mihály Kovács, LMP

Leroy Hood, M.D., Ph.D.,
President, Institute for Systems Biology

10:45 - 11:00 Coffee Break

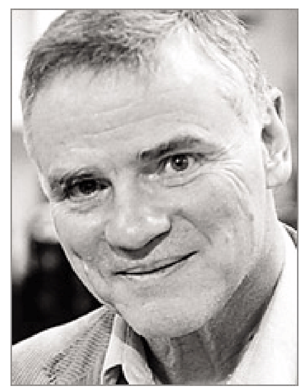
11:00 - 1:00 **Career Development Panel** - Prospect Bay Ballroom
Moderators: Drs. Michael Adams, LMI and Rebecca Lipsitz, LB
Minoo Battiwalla, M.D., BMT Program, Department of Medicine, Roswell Park Cancer Institute - Careers in Academia
Frank Walsh, Ph.D., Senior Vice president and head of Discovery Research at Wyeth Pharmaceuticals, Collegeville, Pa. - Careers in Industry
Christine D. Copple, Ph.D. - President, ASM Resources, Inc.- Entrepreneurship
Anne Plant, Ph.D., NIST - Careers in Government

1:00 - 2:30 **Lunch with Speakers**
Dr. Minoo Battiwalla - Chesapeake Lounge
Dr. Frank Walsh - Prospect Bay Ballroom
Dr. Christine D. Copple - Riverview
Dr. Anne Plant - Bayside Deck

2:30 **Departure**

Speakers Biographies

Richard P. Lifton, M.D., Ph.D., is the Chairman, Department of Genetics, and Professor of Medicine (Nephrology), Genetics & Molecular Biophysics & Biochemistry at Yale University and an Associate Investigator, Howard Hughes Medical Institute. He is also the Chairman, Scientific Advisory Board, CuraGen Corporation. In 1999, he won the The Novartis Award For Hypertension Research of the American Heart Association. He received his B.A. in Biology, from Dartmouth College in 1975 and his M.D. in 1982 and Ph.D. in Biochemistry in1986 from Stanford University School of Medicine, Palo Alto, CA. His laboratory has been identifying genes that contribute to development of vascular disease in humans and determining how these mutations alter normal physiology to result in clinical disease. His work is suggesting new ways of detecting and treating people susceptible to cardiovascular disease. Many of the common diseases encountered in clinical medicine, such as coronary artery disease, stroke, diabetes, asthma, hypertension, and renal disease, are known to have strong genetic components, indicating that inheritance of genetic variants contributes to their occurrence. With the advent of new tools in molecular genetics we now have the capability to identify these susceptibility loci. This identification can provide new insight into disease pathophysiology, provide new tests capable of identifying individuals with inherited susceptibility at pre-clinical stages, and ultimately lead to new therapies tailored to individuals with underlying primary abnormalities.



Leroy Hood, M.D., Ph.D. founded the Institute for Systems Biology, where he serves as president and director, to pioneer systems approaches to biology and medicine. He has also played a role in founding several biotechnology companies, including Amgen, Applied Biosystems, Systemix, Darwin, Rosetta, and MacroGenics. He earned an M.D. from Johns Hopkins University in 1964 and a Ph.D. in biochemistry from the California Institute of Technology in 1968. Hood has published more than 600 peer-reviewed papers and co-authored textbooks in biochemistry, immunology, molecular biology and genetics. He also co-edited "Code of Codes," a book discussing scientific, social and ethical issues raised by genetic research. His professional career began at Caltech, where he and colleagues pioneered, the DNA and protein synthesizers and sequencers that constitute the technological foundation for contemporary molecular biology and that have revolutionized genomics by allowing the rapid automated sequencing of DNA. An early advocate of the Human Genome Project, he also played a pioneering role in deciphering the secrets of antibody diversity. He is a member of the National Academy of Sciences, the American Philosophical Society, and the American Association of Arts and Sciences. His many awards and honors include the Lasker Award in 1987 for studies on the mechanism of immune diversity, the Kyoto Prize for Advanced Technology in 2002, and the Lemelson-MIT Prize for Invention and Innovation in 2003. In addition to his many other achievements in research and business, he has maintained a life-long commitment to making science accessible and understandable to the general public, especially children.

Minoo Battiwalla, M.D., is a member of the Leukemia Section in the Department of Medicine at Roswell Park Cancer Institute. Dr. Battiwalla joined the faculty of Roswell Park Cancer Institute in 2003 and was appointed to the Leukemia Section of the Department of Medicine. Dr. Battiwalla came to RPCI from the National Institutes of Health, where he completed a four-year fellowship in Hematology and Medical Oncology. He earned Bachelors of Medicine and Surgery degrees at the Medical College, Calcutta, India, in 1994 and completed residency training in Internal Medicine at the University Hospital at Stony Brook, NY, in 1999. He also earned a Master's degree in Clinical Research from the Duke University School of Medicinee. Dr. Battiwalla's research interests include malignant hematology and stem cell transplantation, with a specific focus on the effects of the immune system in leukemia and myelodysplastic syndromes and the development of approaches to reducing the toxicity of stem cell transplantation. Dr. Battiwalla is a Diplomate in the American Board of Internal Medicine and the subspecialty Boards of Oncology and Hematology. He is a member of the American Society of Hematology, American Society of Blood and Marrow Transplantation and the American College of Physicians. Dr. Battiwalla has authored or co-authored several journal articles, abstracts and book chapters.

Christine Copple, Ph.D., is President and CEO of ASM Resources, Inc. Dr. Copple received her B.S. degree from the University of East Anglia and her doctorate from the University of Birmingham Medical School in the United Kingdom. She came to the U.S. to join the Cell Biology Laboratory at Cold Spring Harbor Laboratory on Long Island, New York. With over twenty years as a senior executive in biotechnology growth companies Dr. Copple has experience in public offerings, private/venture investments, joint ventures, licensing and partnering, public corporate reporting, public relations, investor relations, international marketing, product development, regulatory affairs, legislative affairs, and facilities design/build-out. Chris was the Chief Operating Officer at Neuralstem, Inc., an emerging company commercializing a CNS stem cell technology platform. Before joining Neuralstem she ran the Office of Industry Liaison for the University of Maryland Biotechnology Institute where she provided entrepreneurial guidance and services for faculty and start-up companies. For the ten years prior to relocating to Maryland, Chris was V.P. of Marketing & Investor relations for the Boston public company Microfluidics International.

Anne L. Plant, Ph.D. is a member of the Cell & Tissue Measurements Group at the National Institute of Standards and Technology in Gaithersburg, MD. She received her Ph.D. in Biochemistry in 1984 from the Baylor College of Medicine, Houston, TX, followed by an NRC Associateship at the Optical Sciences Group of the Naval Research Laboratory. During the period 2003-2005 she was a Staff member at the Office of Science and Technology Policy, of the Executive Office of the President, after which she returned to NIST. Her current research interests are quantitative cell biology using fluorescence microscopy; controlled surface chemistries at the micro- and nano-scale for studies of cell response to extracellular matrix proteins; the structure and function of biological membranes and membrane-bound proteins; biophysics of model membranes that have potential application as sensor, medical diagnostics, and research tools; and biomolecular interactions at surfaces.

Frank S. Walsh, Ph.D., F.Med.Sci. is Executive Vice President, Discovery Research Worldwide, Wyeth Pharmaceuticals. Dr. Walsh received his Ph.D. in Biochemistry from University College, London, in 1977. He then undertook a one-year post doctoral research fellowship at NHLBI with Dr. Marshall Nirenberg, returning to the UK in 1979 to the Institute of Neurology in London. In 1989, Frank moved to the United Medical and Dental Schools of Guy's and St. Thomas's Hospitals (UMDS), London, becoming the Sir William Dunn Professor of Experimental Pathology, and later served as the UMDS's Research Dean. In 1997, Dr. Walsh moved to SmithKline Beecham (SB) Pharmaceuticals at Harlow, UK, to become Vice President and Director of Neuroscience Research. With the creation of GSK from the merger of SB and GW, Frank became Senior Vice President and Head of the Company's Neurology-CEDD. In 2002, Dr. Walsh moved to Wyeth Research, Collegeville, Pennsylvania, USA, to become Senior Vice President and Head of Discovery Research. In January, 2005, he was promoted to Executive Vice President, Discovery Research Worldwide. Dr. Walsh serves on a number of advisory boards including those of the UK Medical Research Council's Centre for Developmental Neurobiology; the ALS Research Center at Johns Hopkins University, Baltimore, USA; the UK Muscular Dystrophy Campaign; and the CEO Council of the New York Academy of Sciences. He holds Visiting Professorships at London University's King's College, the University College Dublin, and was elected to the Academy of Medical Sciences (London) in 2003. In October of 2004, he was awarded an Honorary Degree "Laurea Honoraris Causa," in Chemistry and Technology of Drugs from the University of Perugia, Italy. He is currently Chief Editor of the journal, Molecular and Cellular Neuroscience.

NHLBI Core Facilities and Services

Bioinformatics Core Facility

Eric Billings, Ph.D., Director
Building 10, Room 4A15, E-mail: billingse@nhlbi.nih.gov
Phone: (301) 496-6520, Fax: (301) 480-1243

The NHLBI Bioinformatics Core Facility is chartered to facilitate intramural scientists' research by providing access to existing and new tools for both discovery and hypothesis driven research. The core will support advanced users with application software; provide training for new and advanced users; facilitate access to Affymetrix and new spotted array scanners; field general questions about Bioinformatics tools; provide collaborative research support with post-doctoral bioinformatics staff; provide easy access to in-house workstations loaded with bioinformatics research tools. In the long term, nn integrated approach to genomics and proteomics is being developed within the Core Facility. Academic, commercial and open source approaches are being combined to provide a platform for addressing scientific questions that span genomic, proteomic and other information sources.

Electron Microscopy Core Facility

Zu-Xi Yu, M.D., Ph.D., Facility Manager:
Building 10, Room 2N246, E-mail: yuz@helix.nih.gov
Phone: (301) 402-0908, Fax: (301) 402-4127

The Core Electron Microscopy Facility at NHLBI provides contemporary transmission electron optical services to all investigators within the Institute. Services include standard light microscopy, transmission electron microscopy of cells and tissues, immuno-electron microscopy (immunocytochemistry at the electron microscopic level, including immuno-gold labeling) on ultrathin plastic or frozen sections, negative staining or rotary shadowing of macromolecule preparations (nucleic acids or proteins), autoradiography at the light and electron microscopic level, computer-assisted morphometry and image analysis, diagnostic pathology at both histological and ultrastructural level, and full range of traditional dark room services.

Flow Cytometry Core Facility

J. Philip McCoy, Jr., Ph.D., Director
Building 10, Rooms 4A07 and 4A11, E-mail: mccoyj@nhlbi.nih.gov
Phone: (301) 594-6950, Fax: (301) 480-4774

The mission of the NHLBI Flow Cytometry Core Facility is to provide investigators at the NHLBI access to state-of-the-art flow cytometry. This is done by having cytometers and software available in the core facility and by providing consultation to investigators who have cytometers available in their own laboratories or branches. Investigators are responsible for specimen preparation and staining. The staff of the flow cytometry laboratory will gladly assist you in designing your experiments and in developing optimal preparation and staining procedures. For analytical experiments, data will be provided as either hard copies or on appropriate media as listmode files. Summit software (Cytomation, Inc) will be available for "offline" analysis of these files. For sorting experiments, each investigator is responsible for bringing appropriate media and test tubes.

Light Microscopy Core Facility

Christian A. Combs, Ph.D., Facility Director
Building 10, Room 5D19; E-mail: combsc@nhlbi.nih.gov
Pager: (301) 930-3020

The mission of the light microscopy core facility is to provide state of the art equipment, training, and image processing capabilities to assist researchers within the NHLBI-DIR in experiments involving light microscopy. Equipment within the facility includes several types of confocal microscopes, a two-photon microscope, and a standard epi-fluorescence widefield microscope. This range of instruments provides capabilities that include live cell imaging, deep tissue-level imaging, video-rate confocal imaging, spectral imaging, and simple widefield fluorescence and brightfield imaging of prepared slides Image processing capabilities include deconvolution, digital linear unmixing of spectrally overlapping fluorochromes, and 3D reconstruction as well as a custom in-house image processing programs for specific applications. It is intended that this webpage provide researchers with all of the information necessary to plan their experiments based on the capabilities of the core instruments as well as to provide background information on the light microscopy techniques that are available.

Office of Biostatistics Research (OBR)

Nancy L. Geller, Ph.D., Director
Rockledge2, Room 8210; E-mail: gellern@nhlbi.nih.gov
Phone: (301) 435-0434

The OBR collaborates in the planning, design, implementation, monitoring and analyses of studies funded by NHLBI. OBR also provides statistical consultation to any NHLBI investigator who requests advice and collaborates in data management and analysis of some studies sponsored by the Division of Intramural Research. The professional staff is often asked to serve on in-house administrative committees as well as advisory committees for other Institutes within NIH and other agencies within DHHS. OBR’s primary responsibility is to provide objective, statistically sound, and medically relevant solutions to problems that are presented. When a question raised requires new methodology, the OBR is expected to obtain a new and valid statistical solution.

Office of Technology Transfer and Development (OTTAD)

Marianne Lynch, J.D., Technology Development Specialist
Rockledge1, Room 6018; E-mail: lynchm@nhlbi.nih.gov
Phone (301) 594-4094; Fax: (301) 594-3080

OTTAD provides a complete array of services to support the National Heart, Lung, and Blood Institute's technology development activities. To ensure that these activities comport with Federal statutes, regulations and the policies of the National Institutes of Health, a large part of OTTAD's responsibilities includes the day-to-day negotiations of transactional agreements between the NHLBI and outside parties, including universities, pharmaceutical and biotechnology companies. These agreements provide for:

- The exchange of research materials under the Simple Letter of Agreement (SLA);
- Collaborative research conducted under cooperative research and development agreements (CRADAs);
- Preclinical and clinical studies of the safety and efficacy of new pharmaceuticals under clinical trial agreements (CTAs); and
- Exchange of confidential information under confidential disclosure agreements (CDAs).

The OTTAD also reviews employee invention reports, generates patentability reports and makes recommendations to the NIH's Office of Technology Transfer (OTT) concerning filing of domestic and foreign patent applications. The NHLBI OTTAD staff participates in meetings, discussions and conferences, as appropriate, to stay apprised of and monitor the scientists' needs.

Pathology Core Facility

Zu-Xi Yu, M.D., Ph.D., Facility Head
Building 10, Room 2N240; E-mail: yuz@helix.nih.gov
Phone: (301) 402-0908, Fax: (301) 402-4127

The Pathology Core is a Morphology Core Facility, which provides histopathological, immunocytochemical, and ultrastructural support for NHLBI intramural research. The Core Facility provides quality control for morphologic studies, experimental pathology (animal models) and optimizes use of supplies and equipment for all investigators at the NHLBI in which morphological studies and tissue-based molecular studies play a critical role. Ongoing interaction of Pathology Core personnel with each investigator facilitates communication regarding morphologic findings, histopathological interpretation, and new technical developments, thus increasing the efficiency of the research projects. Staff are well-trained, extremely experienced technicians, and the laboratory has a wide repertoire of specialized techniques. The research pathology and immunohistochemistry are subsequently operating using standard operating procedures based on good lab practice guidelines.

Proteomics Core Facility

Rong-Fong Shen, Ph.D., Chief
Bldg. 10, Room 6C208; E-mail: ShenR@nhlbi.nih.gov
Phone: (301) 594-1060, Fax: (301) 402-2113

The mission of the Core is to facilitate investigators in the NHLBI's Division of Intramural Research accomplish their proteomics endeavors by providing mass spectrometry-based analyses of samples. The Core maintains state-of-the-art mass spectrometers, provides guidance to sample preparation, trains scientists and research fellows for the use of instrument and data analysis, and develops analytical methodologies relevant to proteomics.

Transgenic Mouse Core Facility

Chengyu Liu, Ph.D., Chief
Building 50, Room 3537; E-mail: Liuch@nhlbi.nih.gov
Telephone: (301) 435-5034, Fax: (301) 435-4819

The NHLBI Transgenic Facility is a state-of-the-art transgenic mouse laboratory established in 1999. Its main function is to assist NHLBI scientists making transgenic and knockout mice. Generally, the users of the facility are responsible for making the DNA constructs, and screening the potential positive ES cell clones and founder mice. The staff at the facility are responsible for the culturing, transfecting, and selecting ES cells, as well as microinjecting and implanting mouse embryos. After creation, the transgenic or knockout mice are transferred to each users' animal room for phenotypic analysis. We use the standard pronuclear microinjection method to make transgenic mice. The users are responsible for making the DNA constructs and purifying the fragments for microinjection. Generally, the transgene needs to be separated from the cloning vector and carefully purified before submitting to our facility. Our standard strain of mice is B6CBAF1/J (C57BL/6J x CBA/J). We can also make transgenic mice using FVB/NJ and C57BL/6J inbred strains if necessary. For the production of knockout mice, the users are responsible for making the targeting constructs and providing us with at least 100 microgram of linearized, highly intact DNA (freshly prepared and digested, or store in 70% ethanol at -20˚C). The DNA samples will be electroporated into embryonic stem (ES) cells. The correctly-targeted ES cell clones will be microinjected into blastocyst stage embryos (blastocyst microinjection) to create chimeric mice. At appropriate age, the visibly good chimeric mice will be mated with C57BL and 129 mice to pass the mutated gene to the next generation. Our facility is equipped with microscopes and manipulators that are suitable for dissecting and micro-manipulating mouse embryos and cultured cells. We are also interested in in vitro differentiation of the ES cells through the formation of embryoid bodies (EB).

Poster Session Titles and Assignments

Posters are put up in the morning

Authors of odd numbered posters present 5:00-6:15

Authors of even numbered posters present 6:15-7:30

Biochemistry & Signal Transduction

1. Ang II-induced superoxide generation. H. Choi, L. Hunyady, K.J. Catt, T. L. Leto, Y.S. Bae, and S.G. Rhee; Laboratory of Cell Signaling.

2. Computational Studies of Chaperonin-mediated Protein Folding. G. Stan[1], B. R. Brooks[1], D. Thirumalai[2], and G. H. Lorimer[2], Laboratory of Computational Biology, [1]National Heart, Lung and Blood Institute; [2]Institute for Physical Science and Technology and Department of Chemistry and Biochemistry, University of Maryland.

3. Effect of Bicarbonate on Iron-mediated Oxidation of LDL. H. Arai, B. S. Berlett, P. B. Chock, and E. R. Stadtman; Laboratory of Biochemistry.

4. E3 ubiquitin ligase activity of the trifunctional ARD1 (ADP-ribosylation factor domain protein 1). A. Vichi, D. M. Payne, G. Pacheco-Rodriguez, J. Moss, and M. Vaughan; Pulmonary-Critical Care Medicine Branch.

5. Glucocorticoids Suppress Mast Cell Activation by Different but Complementary Mechanisms. F.A. Lisboa, M.V. Andrade, Z. Peng, T. Hiragun, and M.A. Beaven; Laboratory of Molecular Immunology.

6. High-resolution Mapping of Genome-wide Histone Methylation. S. Cuddapah, T-Y. Roh, K. Zhao; Laboratory of Molecular immunology.

7. Increased Energy Dissipation in Cyclic Nucleotide Phosphodiesterase 3B (PDE3B) Knockout Mice. Y. Tang[1], O. Gavrilova[2], Y. Choi[1], S. Hockman[1], E. Billings[3], V. Manganiello[1]. [1]Pulmonary Critical Care Medicine Branch, [2]National Institute of Diabetes Digestive Kidney Diseases, [3]Bioinformatics Core Facility.

8. Load-dependent Kinetics of Non-muscle Myosin II. M. Kovacs, F. Wang, and J. R. Sellers; Laboratory of Molecular Physiology.

9. Mutation of the Adenylylated Tyrosine of Glutamine Synthetase Alters its Catalytic Properties. S. Luo, G. Kim, R. L. Levine; Laboratory of Biochemistry.

10. PPAR Agonist Pioglitazone Restores Impaired Mitochondrial Biogenesis and Function in a Cell Model of Insulin Resistance. I. Pagel, and M.Sack; Cardiovascular Branch.

11. Selective Hepatic Expression of ABCA1 in ABCA1-KO Mice Identifies Liver ABCA1 as the Major Source of Plasma HDL-C. L. Liu, C. Joyce, B. Vaisman, J. Fruchart-Najib, S. Santamarina-Fojo, B. Brewer; Molecular Disease Branch.

12. The cardiac-specific homeodomain Nkx2.5: Conformational stability and specific DNA binding of Nkx2.5(C56S). E. Fodor and A. Ginsburg; Laboratory of Biochemistry.

Cell Biology

13. Cooperative Activity of BRG1 and Z-DNA Formation in Chromatin Remodeling. H. Liu, N. Mulholland, and K. Zhao; Laboratory of Molecular Immunology.

14. Curing of [PSI+] Yeast Cells by Hsp104 Inactivation Does Not Require Cell Division. Y. -X. Wu[1], L. E. Greene[1], D. C. Masison[2], and E. Eisenberg[1]; [1]Laboratory of Cell Biology, [2]Laboratory of Biochemistry and Genetics, NIDDK.

15. Drosophila Myosin V: Solution Kinetics and Motile Properties. J. Toth, M. Kovacs, F. Wang, J.R. Sellers; Laboratory of Molecular Physiology.

16. Identification and characterization of a novel bifunctional protein in the ER: Calisomerin. D. Y. Lee, W. J. Jeong, T. S. Chang, and S. G. Rhee; Laboratory of Cell Signaling.

17. Interaction of BIG2, a brefeldin A-inhibited guanine nucleotide-exchange protein, with exocyst protein Exo70. K.F. Xu, X. Shen, H. Li, G. Pacheco-Rodriguez, J. Moss, and M. Vaughan; Pulmonary-Critical Care Medicine Branch.

18. Intracellular Translocation of BIG1 and ARF in Response to Cyclic AMP. C. Citterio, and M. Vaughan; Pulmonary-Critical Care Medicine Branch.

Growth, Development, and Aging

19. Adult Murine Skeletal Muscle Contains Cells That Can Differentiate into Neuronal-like Cells. Y. A. Kassahun, S.O. Winitsky, S. Hassanzadeh, H. Takahashi, T.V. Gopal, and N.D. Epstein; Laboratory of Molecular Cardiology.

20. Chondroitin Sulfate Proteoglycan Glycosaminoglycan Chain Synthesis: A Potential Molecular Target. T. Laabs[1,2], J. W. Fawcett[2], and H. M. Geller[1], [1]Developmental Neurobiology Group, [2]Cambridge University Centre for Brain Repair, Cambridge, UK.

21. Effect of Disruption of Caspase-like Gene, YCA1, of Sacchromyces Cerevisiae on Oxidative Stress and Apoptosis Exerted by Hydrogen Peroxide. M. A. S. Khan and E. R. Stadtman; Laboratory of Biochemistry.

22. ENU Induced Mutations Causing Congenital Cardiovascular Anomalies. Q. Yu, Y. Shen, B. Chatterjee, B. Siegfried, L. Leatherbury[1], C. Spurney, Y.J. Wu, C.W. Lo; Laboratory of Developmental Biology.

23. Expression of SUMO-2/3 induced cellular premature senescence and polysumoylation of p53. T. Li[1], R. F. Shen[2], R. Santockyte[3], D. C. H. Yang[3], P. B. Chock[1]; [1]Laboratory of Biochemistry, [2]Proteomic Core Facility, [3]Chemistry, Georgetown University, Washington, DC, 20057.

24. Novel Defects in the Heart Following Conditional Ablation of Mouse Myosin II-B. A. Singh, Q. Wei, K. Takeda, C. Liu, Z. Yu, and R. Adelstein; Laboratory of Molecular Cardiology.

25. Spatial and Temporal Expression Pattern of Kis During Mouse Development. M. Olive, M. Boehm, M. F. Crook, X. Qu and E. G. Nabel; Cardiovascular Branch.

26. Sulfation Is Responsible for Altered Axonal Growth on Astrocytes. H. Wang, Y. Katagiri, H. M. Geller; Developmental Neurobiology Group.

Injury/Inflammation/Immunology

27. A Comparison of Susceptible and Resistant Mice to Drug-Induced Liver Disease Using Genomics, Proteomics and SNPs Analyses. K. Welch, B. Wen, Y. Guo, M. Adams, T. Reilly, D. Goodlett, E. Yi, H. Lee, M. Radonovich, C. Pise-Masison, T. Hays, M. Bourdi, G. Peltz, J. Brady, S. Nelson, and L. Pohl; Laboratory of Molecular Immunology.

28. CD146 (Mel-CAM), an Adhesion Marker of Endothelial Cells, is Expressed on Lymphocyte Subsets in Normal Peripheral Blood and Upregulated by in vitro Activation. M. F. Elshal[1], S. S. Khan[2], Y. Takahashi[3], M. A. Solomon[2], and J. P. McCoy, Jr[1]. [1]Flow Cytometry Core Facility, [2]Critical Care Medicine Department, [3]Hematology Branch.

29. Characterization of Tumor Necrosis Factor Receptor 1 (TNFR1) Vesicles in Human Plasma. J. Zhang, F. I. Hawari, B. Shamburek, B. Adamik, M. Kaler, F. N. Rouhani, and S. J. Levine; Pulmonary Critical Care Medicine Branch.

30. FOS as a Novel Marker of Atherosclerosis. W. D. Patino, O. Y. Mian, J. G. Kang, S. Matoba, L. D. Bartlett, B. Holbrook, H. H. Trout III, L. Kozloff, and P. M. Hwang; Cardiovascular Branch.

31. Genomic Identification and Evaluation of Lipocalin-2 as a Potential Protective Factor in the Inhibition of Acetaminophen-induced Hepatotoxicity by N-acetyl-l-cysteine. M. L. Adams[1], M. F. Radonovich[2], J. N. Brady[2], and L. R. Pohl[*1]; [1]Laboratory of Molecular Immunology & [2]Virus Tumor Biology Section, NCI.

32. NEFA, An ARTS-1 Interacting And Calcium Binding Protein, That Promotes The Release Of Soluble TNFR1. A. Islam and S. J. Levine; Pulmonary-Critical Care Medicine Branch.

33. The Hepatoprotective Role of Interleukin-13 in Drug-induced Liver Disease. S. B. Yee, M. Bourdi, and L. R. Pohl; Laboratory of Molecular Immunology.

Techniques/Imaging

34. Characterization of the Product of Hydroethidine and Superoxide: Intracellular Superoxide Detection by HPLC and Limitations of Fluorescence. H. Zhao, H. M. Fales, E. A. Sokoloski, R. L. Levine, J. V. Vivar, and B. Kalyanaraman; Laboratory of Biochemistry.

35. Emission Ratiometric Determination of Cellular pH by 2-photon Microscopy of 2,3-dicyanohydroquinone and 2-photon Microscope Emission Spectral Calibration. P. D. Jobsis, C. A. Combs, and R. S. Balaban; Laboratory of Cardiac Energetics.

36. Improved Fluorophore-assisted Carbohydrate Electrophoresis (FACE) Analysis of Glycosaminoglycans. X. Wen, H. M. Geller, and Y. Katagiri; Developmental Neurobiology Group.

37. Fabrication of Selenophosphate Synthetase (SPS) Affinity Column for Monitoring Protein Interactions. M. Galloway and T. C. Stadtman; Lab of Biochemistry.

38. Secondary Optical Inner Filter Effects on Two-Photon Excitation Fluorescence Microscopy of NAD(P)H in Mouse Skeletal Muscle, In Vivo. E. Rothstein and R. Balaban; Laboratory of Cardiac Energetics.

Gene Therapy

39. AMD3100 Mobilizes HSC with Unique Characteristics and Long-term Repopulating Potential in Rhesus Macaques. A. Larochelle, A. Krouse, D. Orlic, R.E. Donahue, C.E. Dunbar, and P. Hematti; Hematology Branch.

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42. A blood pressure-genotype interaction in CORIN, the pro-ANP convertase, is associated with an increased risk of LVH in untreated hypertension. J. E. Rame[2,4], M. H. Drazner[1], W. Post[4], J. Lima[4], S. Knappe[3], Q. Wu[3], D. L. Dries[1];[1]Donald W., Reynolds Cardiovascular Clinical Research Center, Division of Cardiology, University of Texas, Dallas, TX, [2]Cardiovascular Branch, National Heart, Lung and Blood Institute, [3]Department of Cardiovascular Research, Berlex Biosciences, Richmond, CA, [4]Division of Cardiology, Johns Hopkins Medical Center, Baltimore, MD.

43. Carotid Atherosclerosis Quality and Quantity, C-reactive protein and Future Cardiovascular Disease: The Cardiovascular Health Study. J. Cao, A. Arnold, T. Manolio, B. Psaty, C. Hirsch, J. Polak, L. Kuller and M. Cushman; Laboratory of Cardiac Energetics.

44. Ex vivo and in vivo RCAS retroviral Integration Profile in A Rhesus Macaque Transplantation Model. J. Hu and C. E. Dunbar, Hematology Branch.

45. Local Delivery of Bone Marrow Stromal Cells into Ischemic Muscle Augments Perfusion via Paracrine Mechanisms Rather than Endothelial Differentiation. A. Wragg, C. Graham, M. Konoplyannikov, M. Ma, S. Ding, and M. Boehm; Cardiovascular Branch.

Abstracts In Alphabetical Order of First Author

Genomic Identification and Evaluation of Lipocalin-2 as a Potential Protective Factor in the Inhibition of Acetaminophen-induced Hepatotoxicity by N-acetyl-l-cysteine. M. L. Adams[1], M. F. Radonovich[2], J. N. Brady[2], and L. R. Pohl*[1]; [1]Laboratory of Molecular Immunology & [2]Virus Tumor Biology Section, NCI.

Drug-induced liver disease (DILD) is a poorly understood phenomena that causes significant economic and health consequences. One major limitation in studying DILD is the lack of animal models that predict toxicity or allow for further mechanistic study. Because of its reliable liver toxicity in animals, acetaminophen (APAP) is often used as a model hepatotoxicant to identify susceptibility and protective factors for DILD. APAP-induced hepatotoxicity is clinically treated with N-acetyl-L-cysteine (NAC), often called an antidote to APAP. Although the primary function of NAC treatment is thought to be its conversion to glutathione, this study examines an additional protective mechanism of NAC. The time-dependent genome-wide changes in hepatic mRNA expression of C57Bl/6J mice treated with APAP only (300 mg/kg, i.p.), NAC only (450 mg/kg, i.p.) or NAC 2 or 3h after APAP were investigated using the Affymetrix Murine Genome U74v2 Set GeneChip® Array. Dramatic increases in mRNA expression of the acute phase protein, lipocalin-2 (LCN2), were observed after NAC treatment either alone or as a treatment after APAP dosing. These increases in LCN2 mRNA were paralleled by increases in LCN2 protein in the liver observed by Western blot analysis and immunohistochemical analysis which showed significant levels of LCN2 in damaged regions of the liver. Since LCN2 has recently been shown to protect the kidneys of mice from ischemia/reperfusion injury, it is possible that this acute phase protein may also have a role in the NAC-induced protection against APAP-induced hepatotoxicity.

Effect of Bicarbonate on Iron-mediated Oxidation of LDL. H. Arai, B. S. Berlett, P. B. Chock, and E. R. Stadtman; Laboratory of Biochemistry.

Oxidation of low-density lipoprotein (LDL) may play an important role in atherosclerosis. In this study, we compared the effects of bicarbonate/CO_2 and phosphate buffer systems on iron-catalyzed oxidation of LDL to malondialdehyde (MDA) and to protein carbonyl and methionine sulfoxide derivatives. We found that LDL oxidation mediated by Fe(II) with H_2O_2 is significantly enhanced in the bicarbonate/CO_2 buffer system relative to those observed with the phosphate buffer. The differential between the two buffer systems is most pronounced with MDA formation in the presence of ascorbate. In addition, when chelators, such as EDTA and ADP, are present in equal molar amounts with iron, the rates of LDL oxidation by Fe(II) with H_2O_2 were also increased for reactions carried out in either the phosphate or bicarbonate/CO_2 buffer. When iron was bound to a chelator, such as cytochrome c or protoporphyrin (hemin), LDL oxidation was significantly stimulated in the bicarbonate/CO_2 buffer. Results of these studies suggest that interactions between bicarbonate and iron or heme derivatives leads to complexes that have redox potentials that favor the generation of reactive oxygen species and/or to the generation of highly reactive carbon dioxide or bicarbonate radicals that facilitate LDL oxidation.

Carotid Atherosclerosis Quality and Quantity, C-reactive protein and Future Cardiovascular Disease: The Cardiovascular Health Study. J. Cao, A. Arnold, T. Manolio, B. Psaty, C. Hirsch, J. Polak, L. Kuller and M. Cushman; Laboratory of Cardiac Energetics.

Background: Recent evidence links high risk carotid plaque with risk of future myocardial infarction (MI). Cross-sectional data suggests that C-reactive protein (CRP) may be a mediator since higher levels correlated with high risk carotid plaques. We prospectively investigated the roles of carotid plaque characteristics and CRP on risk of cardiovascular disease (CVD) defined as incident stroke, MI and CVD death. Methods: We studied participants of Cardiovascular Health Study, a population based study of men and women >65 years of age and followed for at least 9 years. We studied 5020 individuals without pre-baseline stroke or MI. High risk plaques at baseline were defined by ultrasound as those with markedly irregular or ulcerated surfaces, hypoechoic or heterogenous appearance. Low risk plaques were those without discrete plaque and with a smooth intimal surface. All other plaque types were defined as intermediate risk. The hazard ratios (HR) of incident CVD outcomes were estimated by Cox proportional hazards regression. Results: Compared to low risk plaques, intermediate and high risk carotid artery plaques were independently associated with incident CVD with adjusted HR 1.4 (95% CI 1.2, 1.7) and 1.4 (1.2, 1.7) respectively. The incidence rate (IR) of CVD for any given plaque group was much higher when there was more advanced overall carotid atherosclerosis, measured as higher tertile of carotid intima-media thickness (IMT). The IR was further increased if CRP was > 3 mg/L (table). In the presence of higher IMT and intermediate or high risk plaque, those with higher CRP had a 6-7%/year rate of CVD events. Conclusion: Advanced carotid plaque was an independent risk for a future CVD event and elevated CRP added to this risk. Our results suggest that atherosclerosis quality and quantity may be modulated by the destabilizing effect of inflammation, predisposing to clinical events.

Ang II-induced superoxide generation. H. Choi, L. Hunyady, K. J. Catt, T. L. Leto, Y. S. Bae, and S. G. Rhee; Laboratory of Cell Signaling.

The mechanism responsible for the angiotensin II (Ang II)-induced production of reactive oxygen species in nonphagocytic cells was investigated with HEK293 or CHO cells reconstituted with the angiotensin type 1 receptor (AT1R), NADPH oxidase 1 (Nox1), Nox organizer 1 (Noxo1), and Nox activator 1 (Noxa1). Stimulation of the reconstituted cells with Ang II induced a four- to sevenfold increase in superoxide production relative to the constitutive level mediated by the complex of Nox1, Noxo1, and Noxa1, demonstrating both that Nox1 is activated by cell surface receptor-mediated signaling and that AT1R is coupled to Nox1. Expression of several AT1R mutants showed that interaction of the receptor with G proteins, but not that with ß-arrestin or with other proteins (Jak2, phospholipase C-γ1, SHP2) that bind to the COOH-terminal region of AT1R, was necessary for Ang II-induced superoxide production. Evaluation of the effects of constitutively active subunits of G proteins and of various pharmacological agents suggested that signaling by a pathway comprising AT1R, Gaq/11, phospholipase C-ß, and protein kinase C was largely, but not exclusively, responsible for Ang II-induced activation of the Nox1-Noxo1-Noxa1 complex in the reconstituted cells. A contribution of Ga12/13, phospholipase D, and phosphatidylinositol 3-kinase to Ang II-induced superoxide generation was also suggested, whereas the small GTPase Rac1, Src, and the epidermal growth factor receptor did not appear to participate in this effect of Ang II.

Intracellular Translocation of BIG1 and ARF in Response to Cyclic AMP. C. Citterio, and M. Vaughan; Pulmonary-Critical Care Medicine Branch.

Brefeldin A-inhibited guanine nucleotide-exchange protein 1 (BIG1) activates ADP-ribosylation factor (ARF) GTPases that are essential in the molecular machinery regulating vesicular traffic among intracellular compartments. To understand better BIG1 function, effects of serum starvation and 8-Bromo-cyclic AMP (8-Br-cA) on its distribution in HepG2 cells were investigated using confocal immunofluorescence microscopy and Western blot analyses of subcellular fractions with densitometry. Cells grown with serum had BIG1 and ARF distributed similarly, ca. 70, 25, and 5%, respectively, in cytosol, membranes, and nuclei. By immunofluorescence, BIG1 and ARF were seen in the cytoplasm, co-concentrated only in peri-nuclear structures. After 16h without serum, percentages of BIG1 and ARF were lower in membranes (16 and 18% respectively) and higher in nuclei (10 and 13%). Serum-starved cells incubated with 8-Br-cA showed time- and concentration-dependent movement of BIG1 from membranes to nuclei with maximal increase (28%, $p<0.05$) in nuclei and decrease (4%, $p<0.05$) in membranes after 20 min with 1mM; ARF increase in membranes (32%, $p<0.05$) and decrease in nuclei (10%, $p<0.05$) was maximal at 10 min. Changes in cytosol content were not significant. Effects of 8-Br-cA were prevented by PKA inhibitor H-89 and were not mimicked by 8-Br-cG. By immunfluorescence after 8-Br-cA, BIG1 was seen in nuclei and ARF was still concentrated in the peri-nuclear region. These studies implicate PKA-catalyzed phosphorylation in intracellular translocation of BIG1 and ARF in HepG2 cells.

High-resolution Mapping of Genome-wide Histone Methylation. S. Cuddapah, T-Y. Roh, K. Zhao; Laboratory of Molecular immunology.

In eukaryotes, DNA and histones are organized into nucleosomes and covalent modifications of the DNA or the packaging histones are responsible for transmitting the epigenetic information. While a variety of post-translational histone modifications are involved in transcriptional regulation, methylation of the amino-terminal tails of histones have been implicated in the promoter regulation, heterochromatic repression and DNA methylation. In this study we have mapped the histone methylation in the human genome by using a high-resolution and unbiased genome-wide mapping technique, GMAT that we have developed. GMAT is a combination of serial analysis of gene expression (SAGE) and chromatin immunoprecipitation (ChIP) techniques. The highest levels of methylation are found in the promoter region. While histone acetylation and histone 3 lysine 4 methylation are known to define the active state of gene expression, a comparison of these two epigenetic modifications has revealed interesting variations in the modifications at different loci. Furthermore, this study has provided vital information to investigate the histone methylation induced gene repression, which has not yet been well characterized.

CD146 (Mel-CAM), an Adhesion Marker of Endothelial Cells, is Expressed on Lymphocyte Subsets in Normal Peripheral Blood and Upregulated by in vitro Activation. M. F. Elshal[1], S. S. Khan[2], Y. Takahashi[3], M. A. Solomon[2], and J. P. McCoy, Jr[1]. [1]Flow Cytometry Core Facility, [2]Critical Care Medicine Department, [3]Hematology Branch.

CD146 is a well-described adhesion molecule found on endothelial cells as well as a limited number of other cell types. In the peripheral circulation, CD146 is found on circulating endothelial cells and has been used as a means of isolating these cells. This report, for the first time, describes the expression of CD146 on peripheral blood lymphocytes from healthy donors. In samples from 10 healthy donors, CD146 was found on $CD3^+$ T cells (1.95% +/- 1.03%, $CD19^+$ B cells (0.45% +/- 0.30%), and CD3-$CD56^+$ NK cells (0.09% +/- 0.06%). The expression of CD146 on these cells did not correlate with the expression of markers of activation, such as CD69, CD25, or HLA-DR. Additionally the CD146 positive T cells were not clonal, as determined through the use of anti-Vß reagents. By sorting T and B cells for lack of CD146 expression, it was demonstrated that expression of this surface marker could be induced in vitro by activation of both T and B lymphocytes.

The cardiac-specific homeodomain Nkx2.5: Conformational stability and specific DNA binding of Nkx2.5(C56S). E. Fodor and A. Ginsburg; Laboratory of Biochemistry.

The cardiac-specific Nkx2.5 homeodomain has 73% sequence identity to the vnd (ventral nervous system defective)/NK-2 homeodomain of Drosophila and binds the same specific duplex DNA, containing 5´-CAAGTG-3´ at its core. The thermal unfolding of Nkx2.5(C56S) at either pH 6.0 or 7.4 is a reversible, two-state process with unit cooperativity, as measured by differential scanning calorimetry and far-UV circular dichroism. Addition of NaCl to Nkx2.5(C56S) at pH 7.4 stabilizes the protein and gives a heat capacity change of DCp ~ 1.1 kcal K-1mol-1 for homeodomain unfolding. The quench of the conserved Trp48 in Nkx2.5(C56S) and vnd/NK-2 homeodomains is markedly different, possibly due to Arg instead of His at position 52. Titrations of specific 18 bp DNA with Nkx2.5(C56S) in buffer at pH 7.4 with 100 mM NaCl yield binding constants of 3-6 x 108 M-1 from 10 to 37 ˚C and a stoichiometry of 1:1 for homeodomain binding DNA, using isothermal titration calorimetry. The binding reaction is enthalpically controlled and the temperature dependence of DH gives DCp = -0.18±0.01 kcal K-1 mol-1. This corresponds to 648±30 2 of buried apolar surface upon Nkx2.5(C56S) binding 18 bp DNA. Despite the structural similarities the stability and DNA binding properties of Nkx2.5(C56S) are unlike those of NK-2, but similar to those of the NK-2(H52R) mutant studied earlier [Gonzalez et al. Biochemistry 40, 4923-4031, 2001] also, unbound NK-2 is more flexible than is Nkx2.5.

Fabrication of Selenophosphate Synthetase (SPS) Affinity Column for Monitoring Protein Interactions. M. Galloway and T. C. Stadtman; Lab of Biochemistry.

We describe here the development and characterization of an enzyme affinity column for monitoring protein-protein interactions using selenophosphate synthetase (SPS) as the immobilized protein. The solid matrix used in this study was Sepharose (bead-form of agarose gel) activated with cyanogen-bromide giving a derivative that can be readily coupled to unprotonated aliphatic or aromatic primary amine groups of a protein. SPS is a 37 kDa product of the E. coli selD gene that catalyzes the synthesis of selenophosphate from ATP and selenide (1:1). Previous reports showed that selenium bound to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; EC 1.2.1.12) could be utilized by SPS as substrate in place of millimolar levels of free selenide for in vitro synthesis of selenophosphate. In this case GAPDH, a homotetrameric enzyme (containing 1 bound selenium per subunit), served as a selenium delivery protein. In our current studies, the ability of selenium-GAPDH to bind to the immobilized SPS is determined. The protein elution profile revealed 4 bands with apparent molecular masses of 39 kDa representing the GAPDH subunits.

Ex vivo and in vivo RCAS retroviral Integration Profile in A Rhesus Macaque Transplantation Model. J. Hu and C. E. Dunbar; Hematology Branch.

Retroviral induced insertional mutagenesis has contributed to two instances of T cell leukemia in children enrolled in the otherwise successful French SCID-X1 gene therapy clinical trial, which invigorates a huge effort to access the potential hazard of insertional mutagenesis in gene therapy trials. Genome-wide retroviral insertion site analysis has shown MLV vector and HIV based vector integrate preferentially into gene coding regions, whereas the avian sarcoma leucosis virus (ASLV) has a very different integration pattern, with no bias for inserting in or near genes. This virus is also able to transduce non-dividing cells, and thus ASLV-based vectors might be a promising candidate for future HSC gene therapy clinical trials. To explore this possibility, we used an ASLV vector pseudotyped with an amphotropic MLV envelope produced by transfection of vector and helper components in chicken embryonic fibroblast cells. The resulted RCAS (Replication Competent ALV LTR with a Splice donor) vector is able to stably transfect human cells. We obtained vector titers measured on human HEK-293 cells in the range of 2.1 to 8.9×10^6 cfu/ml for vectors carrying either GFP or neomycin resistance marker genes. We found that these ASLV vectors can stably transduce rhesus macaque as well as human peripheral blood $CD34^+$ hematopoietic progenitor and stem cells at levels of up to 30%. We will now use ASLV-transduced rhesus macaque $CD34^+$ cells for in vivo autologous transplantation to assess long-term stem cell gene transfer efficiency, and then analyze vector insertion sites by LAM-PCR to determine whether this vector system can overcome some of the issues of insertional mutagenesis associated with standard MLV vectors. These studies therefore have important implications for future hematopoietic stem cell gene therapy clinical trials.

NEFA, An ARTS-1 Interacting And Calcium Binding Protein, That Promotes The Release Of Soluble TNFR1. A. Islam and S. J. Levine; Pulmonary-Critical Care Medicine Branch.

Tumor necrosis factor is a pro-inflammatory cytokine that has been implicated in the pathogenesis of asthma, COPD, pulmonary fibrosis, and sarcoidosis. The bioactivity of tumor necrosis factor (TNF) is regulated by soluble TNF receptors that function as TNF binding proteins. ARTS-1 (Aminopeptidase Regulator of Type 1 TNF Receptor Shedding), a type II integral membrane protein expressed by human epithelial vascular endothelial (HUVEC) cells, binds to and promotes the release of soluble type I TNF receptor (sTNFR1). We performed a yeast two-hybrid screen, utilizing the ARTS-1 extracellular domain as bait, and co-immunoprecipitation experiments to identify the calcium binding protein, NEFA (DNA-binding/EF-hand/Acidic amino acid-rich protein), as an ARTS-1 interacting protein. Confocal Immunofluorescence microscopy was used to visualize the co-localization of ARTS-1 with NEFA intracellularly. NEFA-specific siRNA produced a significant 73% decrease in sTNFR1 released into culture supernatants from HUVEC over 24 hours, as compared to cells treated with or without control siRNA. PMA-stimulated soluble TNFR1 release was significantly decreased by 82% in siRNA-treated HUVEC. As NEFA possesses EF-hand calcium binding domains, we also investigated whether intracellular calcium levels modulate soluble TNFR1 release. Ionomycin significantly increased sTNFR1 release by 25%, whereas thapsigargin significantly decreased sTNFR1 release by 49%. This demonstrates an important role for intracellular calcium in the regulation of soluble TNFR1 release, consistent with the involvement of the calcium-binding protein, NEFA. In conclusion, we have identified NEFA as an ARTS-1-interacting protein that promotes the release of soluble TNFR1. We propose that the mechanism by which soluble TNFR1 is generated involves a direct protein-protein interaction between ARTS-1 and NEFA.

Emission Ratiometric Determination of Cellular pH by 2-Photon Microscopy of 2,3-Dicyanohydroquinone and 2-Photon Microscope Emission Spectral Calibration. P. D. Jobsis, C. A. Combs, and R. S. Balaban; Laboratory of Cardiac Energetics.

Commonly used "one-photon" ratiometric emission pH probes, such as SNARF and BCECF, can not generate accurate ratiometric measures of cellular pH with two-photon excitation because of large differences in the two-photon cross-sectional area of their acid and base forms. Therefore, we explored the use of 2, 3-dicyanohydroquinone (DCHQ) as an emission ratiometric probe of pH in vitro and in fibroblast cells using two-photon excitation fluorescence microscopy (TPEFM). In addition, a method for spectrally calibrating the Zeiss LSM510 META was developed. The accuracy of the calibration was established by comparing the emission spectra of reference compounds, in one-photon excitation mode, with the META and a reference spectrofluorometer (Jobin-Yvon). Unlike other ratiometric pH probes, emission spectra of both the acid and base forms of DCHQ were similar with one- or two-photon excitation. A two-photon excitation spectrum revealed that near equivalent amplitude emissions of both the acid (pH 6.0, ~457 nm) and base (pH 9.5, ~489nm) forms of DCHQ were produced using 800nm excitation. These results imply that both acid and base forms of DCHQ must have similar two-photon cross-sectional areas at 800 nm permitting the advantageous use of a ratiometric emission analysis of pH. Calibration curves for pH in vitro and in cells were successfully constructed using the ratio of DCHQ emission at ~460nm and ~512nm, the maxima of the difference spectrum between the two forms of the dye. To our knowledge, DCHQ is currently the only effective emission ratiometric pH indicator for two-photon microscopy and may serve as a useful starting point for the development of other TPEFM ratiometric dyes for quantitative measurement of other cell parameters such as Ca^{2+}, Mg^{2+} or Na^+.

Adult Murine Skeletal Muscle Contains Cells That Can Differentiate into Neuronal-like Cells. Y. A. Kassahun, S. O. Winitsky, S. Hassanzadeh, H. Takahashi, T. V. Gopal, and N. D. Epstein; Laboratory of Molecular Cardiology.

Until recently, it was generally accepted that human central nervous system neurons do not replicate after birth. However, this view has been in flux due to the identification of neural stem cells in the brain. We have isolated stem cells ("SPOC cells") within adult murine skeletal muscle that are multipotential in nature and appear to be able to differentiate in vitro into neuronal-like cells, as confirmed by immunostaining with Tuj-1, an early neuronal marker. In order to determine if a unique cell surface marker exists on these neuronal precursor cells, a monoclonal antibody library was generated by inoculating live spoc cells into a rat and fusing the spleen with myeloma cells. One clone, MSCAb 804, is able to detect spoc cells in situ. We used immunostaining to determine the location of these cells in the adult and embryonic mouse. In adult mouse, 804 antigen was detected in the areas of brain that are now known to contain neural stem cells - the periventricular region, hippocampus, and olfactory bulb. To determine the origin of the 804^+ neural progenitor cells, we studied mouse embryos using immunofluorescence, detecting rare 804^+ cells throughout the embryo (804^+ cells comprise < 0.1% of total embryonic cells), including within organs such as the heart. Moreover, we were able to isolate and culture these cells in vitro after cells dissociated from whole embryos are subjected to MACS (Magnetic Activated Cell Sorting). Long-term cultures to study the ability of these embryonic 804^+ cells to differentiate into neurons are ongoing. We plan to use immounostaing to try to localize the 804^+ cells at their earliest stage in development. The identification of neuronal precursors in embryos and in adult extraneural tissue may provide a potential source of cells that can be used in future studies of neuronal regeneration.

Effect of Disruption of Caspase-like Gene, *YCA1*, of *Sacchromyces Cerevisiae* on Oxidative Stress and Apoptosis Exerted by Hydrogen Peroxide. M. A. S. Khan and E. R. Stadtman; Laboratory of Biochemistry.

In our previous study, we established a notion that inhibition of caspases (apoptosis) by general caspase inhibitor elevates the protein carbonyl in multicelluar eukaryotic system (Khan et al., PNAS 2004). To gain further insights into a potential link between oxidative stress and apoptosis, we chose yeast *Saccharomnyces cerevisiae* as a model for unicellular eukaryotic system. In yeast, programmed cell death has been described recently. *YCA1* gene coding for the yeast metacaspase, a homologue of mammalian caspase, plays a crucial role in the regulation of yeast apoptosis. In this study, we correlate the accumulation of protein carbonyl, and apoptosis in response to the treatment of two different concentration (0.5 and 5 mM) of hydrogen peroxide on wild type *Sacchromyces cerevisiae* and *Δyca1* strains. On one hand, this response showed the protein carbonyl in cells lacking *YCA1* has increased significantly as a result of oxidative stress, in comparison with wild type. On the other hand, as expected, the apoptosis was abrogated in *Δyca1* strain owing to the absence of apoptotic-related *YCA1* gene, whereas wild type underwent apoptosis by showing the externalization of phosphatidylserine and displaying TUNEL-postive nuclei. we also studied the degradation processess involved in the removal of oxidized proteins. Our results revealed a strong relationship between accumulation of protein carbonyl and the disruption of caspase-like gene in yeast, which is solely responsible for the execution of apoptosis. We are the first to provide the experimental evidence that demonstrate the correlation between accumulation of protein carbonyl and the inhibition of apoptosis in both multi- and unicellular eukaryotic organisms.

Load-dependent Kinetics of Non-muscle Myosin II. M. Kovács, F. Wang, and J. R. Sellers; Laboratory of Molecular Physiology.

Molecular motors experience various extents of mechanical load while exerting their motile function. Load dependence of their kinetics is therefore of central importance, both in terms of regulation of their mechanical output and coordination between heads of a supramolecular assembly. Non-muscle myosin II isoforms are filament-forming actin-based motors ubiquitously expressed in vertebrates. After an in-depth characterization of the unloaded kinetic and motile properties of these myosins, we have embarked on an investigation of the load dependence of their functional parameters. One way of gathering such information is to assess the kinetic effect of the intramolecular strain imposed on one head by the other in a two-headed (heavy meromyosin-like myosin) molecule bound to actin via both heads. Our data reveal that the nucleotide (ATP or ADP) binding process is not strain-sensitive in non-muscle myosins, but ADP release exhibits a marked strain effect. Compared to the unstrained value, the ADP release rate constant is accelerated 3-5-fold in myosin heads experiencing an intramolecular strain in the 'forward' direction (i. e. towards the plus end of the actin filament), whereas it is slowed down 8-10-fold by 'backward' strain. This finding is in line with the load-dependent behavior of muscle myosin crossbridges that has been indicated as a molecular basis for the Fenn effect. In cytoskeletal motors such as non-muscle myosin II, however, this feature may be linked to additional functions such as a mechanical response to load and/or changes in intracellular ADP concentration, and load-dependent stalling (a possible passive tension-bearing role) of these myosins.

Chondroitin Sulfate Proteoglycan Glycosaminoglycan Chain Synthesis: A Potential Molecular Target. T. Laabs[1,2], J. W. Fawcett[2], and H. M. Geller[1], [1]Developmental Neurobiology Group, [2]Cambridge University Centre for Brain Repair, Cambridge, UK.

Chondroitin sulfate proteoglycans (CSPGs) are up-regulated in the CNS after injury and have been shown to participate in the inhibition of axon regeneration. The glycosaminoglycan (GAG) chains of these molecules are involved in the inhibition of axon growth and in binding growth factors and other molecules. At present, the expression patterns of chondroitin polymerizing factor (ChPF), a key enzyme for CSPG GAG chain synthesis is not known, and it is also not known if reducing the expression of key CSPG GAG chain synthesizing enzymes will result in increased axonal regeneration after injury. Using conventional RT-PCR, we have shown that ChPF mRNA is present in oligodendrocyte precursor cells, meningeal cells, astrocytes, axon growth-inhibitory astrocytic cell line Neu7, axon growth-permissive astrocytic cell line A7 and embryonic and adult brain. Using quantitative real-time PCR we have shown that ChPF is not up-regulated after treatment of cultured astrocytes with brain injury related cytokines TGF-a or TGF-ß1. ChPF is also not up-regulated in the area surrounding a cortical stab lesion in an adult rat. Using a vector based short hairpin RNA we show the reduction of ChPF mRNA by 60% in Neu7 cells. In parallel, this reduction in mRNA levels decreases the level of CSPG GAG chains in the conditioned media of these cells as soon as three days after transfection. This was shown by western blot with CS-56, an antibody raised against the GAG chains of CSPGs. These results suggest that ChPF is a potential molecular target to decrease CSPG GAG chain synthesis and thus, facilitate axonal regeneration after injury.

AMD3100 Mobilizes HSC with Unique Characteristics and Long-term Repopulating Potential in Rhesus Macaques. A. Larochelle, A. Krouse, D. Orlic, R.E. Donahue, C.E. Dunbar, and P. Hematti; Hematology Branch.

The non-human primate model has proven a valuable in vivo assay for evaluation of hematopoietic stem cell (HSC) based gene transfer methodologies, with implications for human gene therapy clinical trials and basic HSC biology. Using this model, we demonstrated superior retroviral gene marking levels in vivo in animals transplanted with transduced peripheral blood (PB) HSCs obtained after mobilization with a combination of G-CSF and SCF, compared to in vivo gene marking levels using PB HSCs collected after mobilization with G-CSF alone or G-CSF and Flt-3. However, SCF is no longer available for clinical applications in the USA and G-CSF alone has become the preferred mobilizing regimen for clinical transplantation. Recently, AMD3100, a bicyclam antagonist of the chemokine receptor CXCR4, has been noted to induce rapid mobilization of primitive hematopoietic cells in both mouse and human, offering an alternative approach for PB HSCs mobilization. We initiated a study to determine in vivo gene marking efficiency obtained after retroviral transduction of $CD34^+$ cells collected from peripheral of rhesus macaque following mobilization with AMD3100. Peripheral blood (PB) cells were harvested from two macaques one or two hours after administration of a single intravenous dose of AMD3100 1mg/Kg. The animal apheresed one hour after injection of AMD3100 required a second dose of AMD3100 and repeat apheresis to increase the yield of collected PBSC. The harvested cells were enriched for CD34+ cells, and transduced for four days with retroviral vectors containing the neomycin phosphotransferase (neo) gene (G1Na), in retronectin-coated flasks in the presence of SCF, Flt-3, MGDF and protamine. Animals were conditioned with 500cGy daily for two days and cells were infused immediately after transduction. DNA from PB cells collected at the time of recovery and monthly after transplantation was analyzed by semiquantitative PCR. The time of follow-up was 5 months in the first animal and 1 month in the second animal. A total of 3.5×10^7 and 7×10^7 CD34+ cells were obtained after mobilization and apheresis of animal 1 and 2, respectively. Post-transduction total counts were 1.3×10^7

(expansion factor -2.7) and 1.0 X 10^8 cells (expansion factor +1.4), respectively. The time of engraftment (time to reach a neutrophil count greater than 500/uL) was 14 days in the first animal compared to 7 days in the second animal. Level of PB mononuclear cell and granulocyte neo gene marking was 5-10% at the time of engraftment for both animals. In animal 1, the level of marking was 2-5% one month after transplant and remained stable until the fifth month when levels of marking of 1-2% were detected. In animal 2, the efficiency of marking was ---- % one month after transplant. Our data indicate long-term in vivo gene markings, validating the ability of AMD3100 to induce mobilization of true long-term repopulating hematopoietic stem cells. The characteristics of primitive cells mobilized by this agent clearly differ from cytokine-mobilized PBSCs, and may offer insights into the processes of mobilization, and offer advantages for genetic manipulation. In vivo gene marking levels using AMD3100 mobilized PBSC are comparable to in vivo levels previously achieved using PBSC mobilized with a combination of G-CSF and SCF, and better than those obtained using PBSC mobilized with G-CSF alone. AMD3100 mobilized PBSC represent an alternative source of HSC amenable to genetic manipulation with integrating retroviral vectors, with potential applications aimed at improving current human gene therapy protocols and providing new insights in basic hematopoietic stem cell biology. In particular, use could be considered in gene therapy approaches for patients with sickle cell anemia; documented complications have precluded mobilization using G-CSF or G-CSF/SCF in these patients.

Identification and characterization of a novel bifunctional protein in the ER: Calisomerin. D. Y. Lee, W. J. Jeong, T. S. Chang, and S. G. Rhee; Laboratory of Cell Signaling.

The endoplasmic reticulum (ER) plays distinct regulatory roles by virtue of the proteins that reside within the lumen. High concentrations of calcium-binding proteins such as calsequestrin and calreticulin facilitate the storage of calcium ions, which are released into the cytosol in response to extracellular signals. Other luminal proteins, such as protein disulfide isomerase (PDI), mediate proper protein folding through catalysis of disulfide bonds. We identified a novel 432-amino acid residue human ER protein that serves both calcium storage and PDI functions and named it calisomerin. Calisomerin is found in animal cells but not in yeast or higher plant cells. It has an ER retention signal (KDEL-COOH) and three thioredoxin-like domains, each containing a WCXXC motif. In HeLa cells, calisomerin is almost as abundant as calreticulin, the major ER calcium storage protein in non-muscle cells. Calisomerin has a high proportion of acidic amino acids and calorimetric experiments demonstrated its capacity to bind calcium. Recombinant calsiomerin exhibited oxidoreductase activity when tested using insulin as substrate. RNA interference (siRNA) was used to reduce calisomerin levels to < 30% in HeLa and Jurkat T cells, resulting in drastically reduced calcium responses to thapsigargin, caged-Ins 1,4,5-P3, and various agonists known to mobilize intracellular calcium through Ins 1,4,5-P3 production. $[Ca^{2+}]$ER in the siRNA-treated cells was ~ 30% that in control cells. Thus, the attenuated calcium responses in these cells were likely due to decreased calcium storage capacity of the ER. The Ca^{2+} responses were restored by expression of calisomerin in the siRNA-treated cells. The depletion of calisomerin also caused an accumulation of cell surface proteins in the ER, probably because of diminished PDI activity in the ER. These results suggest that calisomerin is a bifunctional protein that contributes to calcium homeostasis and protein folding in the ER.

Expression of Sumo-2/3 Induced Cellular Premature Senescence and Polysumoylation of P53. T. Li[1], R. F. Shen[2], R. Santockyte[3], D. C. H. Yang[3], P. B.Chock[1]; [1]Laboratory of Biochemistry, [2]Proteomic Core Facility. [3]Chemistry, Georgetown University

Modifications of proteins by SUMO-1 regulate transcription, nucleocytoplasmic transport, protein stability and protein-protein interactions. Relatively little is known on the functions or protein substrates of SUMO-2 or SUMO3. Here, stable cell lines of human embryonic kidney cells overexpressing processed forms (SUMO-2/3GG) as well as non-conjugatable forms (SUMO-2/3ΔGG) of SUMO-2 and SUMO-3 were established and analyzed. Cells overexpressing SUMO-2/3GG showed premature senescence phenotype as shown by the cellular morphology and senescence associated galactosidase activity assay. The senescence pathway protein, p21, was up-regulated in cells overexpressing SUMO-2/3GG. In contrast, cells overexpressing SUMO-2/3ΔGG did not show the apparent phenotype or elevated p21. The pleiotropic regulatory protein, p53, was polysumoylated by SUMO-2 and SUMO-3. The K386R (the modification site for SUMO-1) mutant of p53 could not be modified by SUMO-2 or SUMO-3 suggesting that the site of sumoylation on p53 for SUMO2/3 was also at Lys386. Furthermore, when cells were treated with H_2O_2, the modification of p53 by SUMO-2/3 increased, while modification of p53 by SUMO-1 remained unchanged, suggesting possible differential roles among SUMOs. The transcriptional activation by p53 was stimulated when SUMO-2/3GG was overexpressed.

Transducing Efficiency Screening for rAAV2, rAAV1, and rAAV5 in 56 Human Tumor Cell Lines. L. Li, L. Yang, D. A. Scudiero, R. H. Shoemaker, and R. M. Kotin; Laboratory of Biochemical Genetics.

Recombinant adeno-associated viral vectors (rAAV) can efficiently transfer genes to a broad range of mammalian cell types leading to high levels of stable and long-term expression. Using rAAV to deliver therapeutic genes has been highly effective in both research and pre-clinical studies. AAV2 capsids utilize heparan sulfate proteoglycans (HSPG) as a cell surface ligand and possibly fibroblast growth factor1 and alpha V integrins as receptors that mediate cellular entry, whereas other AAV serotypes appear to use different attachment receptors, such as alpha 2-3 O-linked sialic acid and N-linked sialic acid for AAV4 and AAV5 respectively. The receptors and cell surface ligands for other serotypes have not been determined. Therefore, matching a particular rAAV serotype to an appropriate target cell is necessary to achieve efficient transduction. To explore the broad range applications of rAAV delivery in tumor therapy, we analyzed the ability of three diverse AAV serotype vectors to transduce 56 human tumor cell lines. Three different isogenic rAAV serotype vectors with the EGFP reporter gene were produced using AAV2 ITRs and Rep protein, and structural proteins from AAV1, AAV2, and AAV5 respectively, in Sf9 cells by using baculovirus expression vectors. The 56 cell lines are derived from nine distinct tumor types. Tumor cell lines were transduced 24 hr post-plating using high MOI (Multiplicity Of Infection, based on particles per cell) of = 10,000 virus particles per cell. The EGFP transduced cells were observed by fluorescence microscopy and analyzed quantitatively by flow cytometry. Our results showed the majority of melanoma cell lines and glioma cell lines are readily transduced with rAAV. Some lung and breast tumor cell lines have a relatively high rAAV transducing efficiency: the EGFP intensity was about the same as 293 cells, but lower than most of the melanoma cell lines. We found low rAAV transduction for tumor cell lines derived from prostate, renal, and ovarian cancers while most of the cells from cancer of breast, colon, and blood cells (leukemia) were poorly trnansduced with rAAV. Of the three AAV serotypes tested, we found that in general, rAAV2 had the earliest EGFP expression onset. Of the cell types that were transduced with rAAV, the three serotypes the highest transduction rate ranking was observed: AAV2 > AAV5 > AAV1, or AAV5 > AAV2 > AAV1, with some exceptions. These results suggest that AAV vectors have potential use as gene delivery vectors, particularly for melanoma and CNS tumors.

Glucocorticoids Suppress Mast Cell Activation by Different but Complementary Mechanisms. F. A. Lisboa, M. V. Andrade, Z. Peng, T. Hiragun, and M. A. Beaven; Laboratory of Molecular Immunology.

The glucocorticoids inhibit release of cytokines and other inflammatory mediators in mast cells by either transrepression or transactivation of genes. In transrepression monomeric glucocorticoid receptor (GR) interacts with transcription factor complexes to suppress cytokine genes transcription. We find that glucocorticoids also induce transactivation of genes via dimeric GR to increase levels of inhibitory regulators such as the Ras inhibitor, Dok-1, and the Syk inhibitor, SLAP. As a consequence, Ras-dependent signaling pathways, such as ERK 1/2 and Syk-dependent pathways, such as phosphatidylinositol (PI) 3-kinase/PLC /Ca^{2+}, are inhibited. Individually, these pathways regulate production of arachidonic acid and degranulation and, collectively, production of cytokines. Following engagement of the mast cell IgE receptor (FcepsilonRI) by antigen, the subsequent propagation of activating signals is facilitated by migration of FcepsilonRI to lipid rafts and the assembly of signaling molecules with lipid raft-associated adaptors such as LAT. Glucocortiocoids suppress activation of phospholipase D (PLD) as well as PI 3 kinase, which may be critical in this process. We have therefore examined whether suppression of PI 3-kinase and PLD impairs the functional activity of lipid rafts. Indeed, we find that the glucocorticoid, dexamethasone, not only inhibits the migration of FcepsilonRI to lipid rafts, but also causes dispersal of lipid raft markers such as LAT and Thy-1. The apparent disruption of lipid rafts by dexamethasone may further contribute to diminished propagation of signals in activated mast cells.

Cooperative Activity of BRG1 and Z-DNA Formation in Chromatin Remodeling. H. Liu, N. Mulholland, and K. Zhao; Laboratory of Molecular Immunology.

The mammalian genome contains tens of thousands of CG and TG repeat sequences that have high potentials to form the non-classical left-handed double helical Z-DNA structure. Previously we showed that activation of the CSF1 gene by the chromatin remodeling enzyme, BRG1, results in formation of Z-DNA at the TG repeat sequence located within the promoter. In this report, we find that the TG repeats are assembled in a positioned nucleosome in the silent CSF1 promoter and that activation by BRG1 disrupts this nucleosome and results in Z-DNA formation. Active transcription is not required for the formation of Z-DNA, but does results in an expanded region of Z-DNA. Both BRG1 and the Z-DNA forming sequence are required for effective chromatin remodeling of the CSF1 promoter. We show that the interplay of Z-DNA and BRG1 in chromatin remodeling is applicable to more target genes. We propose the Z-DNA formation induced by BRG1 promotes a transition from a transient and partial remodeling to a more extensive disruption of the canonical nucleosomal structure. The data presented in this report establish that Z-DNA formation is an important mechanism in modulating chromatin structure, similar to the activities of ATP-dependent remodelers and posttranslational histone modifications.

Selective Hepatic Expression of ABCA1 in ABCA1-KO Mice Identifies Liver ABCA1 as the Major Source of Plasma HDL-C. L. Liu, C. Joyce, B. Vaisman, J. Fruchart-Najib, S. Santamarina-Fojo, B. Brewer; Molecular Disease Branch.

Recent studies in ABCA1 transgenic and knockout mice support the concept that liver ABCA1 is a major source of plasma HDL-C. To directly evaluate the specific contribution of liver ABCA1 to plasma total and HDL cholesterol we generated transgenic mice that selectively overexpress human ABCA1 in liver and crossed them with ABCA1-KO mice (L-ABCA1-Tg x ABCA1-KO mice). Liver expression of ABCA1 normalized the plasma lipid profile in ABCA1-KO mice. In female ABCA1-KO mice, plasma TC, FC, CE, PL, and HDL-C concentrations were 12+/-0.8, 6.0+/-0.4, 6+/-0.7, 41+/-1.7 and 10+/-0.8 mg/dL, respectively. The corresponding values increased to 55+/-3, 11+/-0.8, 43+/-2.8, 107+/-6.2 and 52+/-4.7 mg/dL for hetero and to 88+/-5, 17+/-1.5, 71+/-3.3, 152+/-7.4, and 81+/-5.2 mg/dL for homo L-ABCA1-Tg x ABCA1-KO female mice. Male hetero and homo mice showed similar dramatic increases in plasma HDL compared to male ABCA1-KO mice. apoA-I was also significantly increased in hetero and homo mice vs ABCA1-KO mice. Plasma lipoprotein analysis by FPLC and native agarose gel electrophoresis confirmed increased plasma HDL-C and alpha-HDL in L-ABCA1-Tg x ABCA1-KO mice. To evaluate the effect of hepatic ABCA1 expression on atherosclerosis L-ABCA1-Tg x ABCA1-KO mice have been placed on a pro-atherogenic diet. In summary: 1) We have generated transgenic mice that selectively express functional human ABCA1 in liver of ABCA1-KO mice. 2) Liver ABCA1 raises plasma total and HDL-C levels, correcting the lipid and lipoprotein abnormalities in ABCA1-KO mice. 3) These findings identify liver ABCA1 as the major source of HDL-C in plasma 4) L-ABCA1-Tg x ABCA1-KO mice will help to define the role of hepatic ABCA1 in atherosclerosis.

Mutation of the Adenylylated Tyrosine of Glutamine Synthetase Alters its Catalytic Properties. S. Luo, G. Kim, R. L. Levine; Laboratory of Biochemistry.

Glutamine synthetase is central to nitrogen metabolism in the Gram negative bacteria. The amount of glutamine synthetase in the cell and its catalytic activity are tightly regulated by multiple, sophisticated mechanisms. Reversible covalent modification of Tyr-397 is central to the regulation of glutamine synthetase activity, via esterification of the hydroxyl group to AMP in a process termed adenylylation. As expected, site-specific mutation of this surface-exposed Tyr-397 to Phe, Ala, or Ser was found to prevent adenylylation. Unexpectedly, these mutations had major effects on the catalytic characteristics of glutamine synthetase. The specific activities of each mutant were approximately doubled, the pH-activity profiles changed, and divalent-cation specificity was altered. Overall, Tyr397Phe behaved as if it were unadenylylated while both Tyr397Ala and Tyr397Ser behaved as if they were adenylylated. Thus, subtle modifications in the environment of residue 397 are sufficient to induce changes previously thought to require adenylylation.

Spatial and Temporal Expression Pattern of Kis During Mouse Development. M. Olive, M. Boehm, M. F. Crook, X. Qu and E. G. Nabel; Cardiovascular Branch.

Progression through G1 phase of the cell cycle and initiation of DNA replication in S phase are regulated by the coordinated activities of the CDKs and their inhibitors, the CKIs. The cyclin-dependent kinase inhibitor, p27Kip1, which regulates cell cycle progression, is controlled by its subcellular localization and subsequent degradation. p27Kip1 is phosphorylated on serine 10 (S10) and threonine 187 (T187). hKIS kinase (human kinase interacting stathmin) is a nuclear protein that phosphorylates p27Kip1 on S10 promoting its nuclear export to the cytoplasm. hKIS is also activated by mitogens during G0/G1 phase of the cell cycle, and expression of hKIS overcomes growth arrest induced by p27Kip1. In order to further understand the function of hKis, we studied the spatial and temporal regulation of Kis during mouse development and compared it with p27Kip1 and proliferation marker PCNA (Proliferating Cell Nuclear Antigen) expression pattern. We characterized their expression patterns using immunohistochemical analysis. Preliminary data suggest that Kis is in derivatives of all three germ layers, the endoderm, mesoderm and ectoderm and in all major organs of the body during key stages of their genesis. It is predominantly expressed in the nucleus. Kis and PCNA

PPAR Agonist Pioglitazone Restores Impaired Mitochondrial Biogenesis and Function in a Cell Model of Insulin Resistance. I. Pagel, and M. Sack; Cardiovascular Branch.

Insulin resistance in skeletal muscle associates with the genomic dysregulation of mitochondrial function, orchestrated, in part via downregulation of the nuclear regulators of mitochondrial biogenesis including peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) and nuclear respiratory factor 1 (NRF-1). These skeletal muscle metabolic and regulatory perturbations presage the development of type 2 diabetes (T2DM). As the thiazolidinediones (TZD's - insulin sensitizers) delay the development of T2DM in insulin resistant individuals, we hypothesized that TZD's may reverse mitochondrial biogenesis dysregulation during insulin resistance. We tested this hypothesis in insulin resistant skeletal myotubes by administering the TZD pioglitazone. C2C12 myotubes were cultivated in medium containing either low glucose (LG, 25mM) or high glucose + high insulin (HGI, 40mM/100nM); vehicle or Pioglitazone (Pio, 50 M) was added for 2 days. C2C12 cells treated with HGI showed blunted activation of insulin target proteins shown as reduced phosphorylation of IRS-1, Akt and ERK, reflecting insulin resistance. In parallel, mRNA of PGC-1, NRF-1 and mtTFA (mitochondrial transcription factor A) were decreased compared to LG conditions, demonstrating a downregulation of the mitochondrial biogenesis program. This genomic phenotype was mirrored by smaller mitochondria, reduced oxygen consumption, decreased mitochondrial membrane potential and diminished ATP content. Treatment with pioglitazone partially restored insulin signaling and normalized mRNA concentration of PGC-1, NRF-1 and mtTFA. In pioglitazone treated cells, reduced oxygen consumption under HGI conditions was normalized as was ATP production. In conclusion, this pioglitazone-mediated normalization of the mitochondrial biogenesis program and associated mitochondrial respiration may provide a mechanism whereby this class of drug delays the development of T2DM.

FOS as a Novel Marker of Atherosclerosis. W. D. Patino, O. Y. Mian, J. G. Kang, S. Matoba, L. D. Bartlett, B. Holbrook, H. H. Trout III, L. Kozloff, and P. M. Hwang; Cardiovascular Branch.

Circulating monocytes mediate inflammation in atherosclerosis and may serve as easily accessible reporters of disease. To search for markers of disease state, we compared the in vivo transcriptomes of monocytes purified from patients undergoing carotid endarterectomy because of atherosclerotic disease and from normal subjects using the serial analysis of gene expression (SAGE) technique. We selected a subset of differentially expressed monocyte-specific genes and prospectively confirmed their expression levels. The Finkel-Biskis-Jinkins osteosarcoma (FOS) gene was significantly increased in patients, and the highest levels of FOS associated with patients who had previously undergone coronary revascularization. The correlation between coronary revascularization and FOS was higher than with the cardiac risk marker high sensitivity C-reactive protein (hsCRP). In vitro manipulation of FOS using siRNA and statins affected monocyte activation, suggesting an important role in pathogenesis. Given the prominent role of FOS in inflammation and calcification, its association with atherosclerosis severity has clear pathophysiologic bases as well as clinical implications as a marker. Our results also suggest that analysis of gene expression in circulating monocytes may provide unique biological and clinical insight into human atherosclerosis.

A blood pressure-genotype interaction in CORIN, the pro-ANP convertase, is associated with an increased risk of LVH in untreated hypertension. J. E. Rame[2,4], M. H. Drazner[1], W. Post[4], J. Lima[4], S. Knappe[3], Q. Wu[3], D. L. Dries[1]; [1]Donald W. Reynolds Cardiovascular Clinical Research Center, Division of Cardiology, University of Texas, Dallas TX, [2]Cardiovascular Branch, National Heart, Lung and Blood Institute, [3]Department of Cardiovascular Research, Berlex Biosciences, Richmond, CA, [4]Division of Cardiology, Johns Hopkins Medical Center, Baltimore, MD.

Background: Left ventricular hypertrophy (LVH) is a known predictor of cardiovascular events, including heart failure. The natriuretic peptide system has been invoked in physiologic counter-regulation promoting vasodilatation, natriuresis, and attenuating cardiac hypertrophy and fibrosis. We hypothesized that individuals who are genotype positive for two novel non-synonymous non-conservative single nucleotide polymorphisms in complete linkage disequilibrium in corin, the pro-ANP/BNP convertase, would be susceptible to the development of LVH as a result of a modification of the left ventricular mass (LVM)-systolic blood pressure (SBP) relation. Methods: Individuals with comparable cardiac MRI measurements to determine LVM in the Dallas Heart Study (DHS) and in the Multi-Ethnic Study of Atherosclerosis (MESA), our replication sample, were genotyped at the T555I/Q568P locus using a TaqMan assay. Multivariate linear regression models were used to determine the presence of an interaction between SBP and corin genotype on LVM in African Americans he group in which the variant was almost exclusively present with a prevalence ~12% ho were not on anti-hypertensive medication in DHS (N=913) and MESA (N=636). Results: A positive and significant interaction between corin genotype and systolic blood pressure on LVM was identified in the DHS (p=0.005) and MESA (p=0.006), independent of relevant covariates and measures used to adjust for body size. Individuals with the corin variant have an excess increase in LVM of 4 grams per 10 mm Hg of SBP when compared to wildtype. A significant increase in prevalent LVH (40.0% versus 18.6%, p=0.01) was also identified in the group of untreated hypertensives who were positive for the corin variant, as compared to group of untreated hypertensives without a copy of the corin minor allele. Conclusion: Strong evidence exists that African-Americans who are genotype positive for the corin variant are more susceptible to developing LVH in the setting of untreated hypertension than their wildtype counterparts. This increased susceptibility may be the result of a modification of the LVM-SBP relation enhancing the hypertrophic response to increased afterload.

Secondary Optical Inner Filter Effects on Two-Photon Excitation Fluorescence Microscopy of NAD(P)H in Mouse Skeletal Muscle, In Vivo. E. Rothstein and R. Balaban; Laboratory of Cardiac Energetics.

The dynamic optical inner-filter effects on emitted light by tissue chromophores must be considered for two-photon excitation fluorescence microscopy (TPEFM) in vivo. While primary optical inner-filter effects (excitation) are minimal in TPEFM, secondary inner-filter effects (emission) have been observed in blue wavelengths in many systems. This study characterized secondary tissue inner-filter effects on NAD(P)H emission from mouse skeletal muscle in vivo. The tissue-dependent spectral modification of TPEFM NAD(P)H emission signal was demonstrated by increasing the amount of tissue between the emission and collection planes by varying the depth of sampling in tissue. These studies revealed a NAD(P)H emission red shift from ~436 (surface) to ~457 nm (70 microns deep), consistent with the tissue absorbance characteristics. To further characterize the tissue absorbance properties, the effect of ischemia/reperfusion on the NAD(P)H emission spectrum (380-700nm) was determined using surface fluorescence (~360nm excitation). The overall NAD(P)H emission intensity with ischemia initially dropped and then increased. With reperfusion, the emission dramatically dropped, then recovered towards control. These global changes were confirmed in TPEFM time courses, in vivo. Spectrally, ischemia was initially associated with a large increase in ~436 nm absorbance, with smaller increases at 500-550 nm, consistent with deoxygenation of myoglobin/hemoglobin (Mb/Hb). After Mb/Hb absorbance stabilized, a large increase ~462 nm signal was observed, consistent with increasing NAD(P)H. Upon reflow, the Mb/Hb absorbance disappeared and the NAD(P)H signal decreased below control, consistent with the post-ischemic activation of aerobic metabolism. These data demonstrate dynamic interference by tissue inner-filters in the visible regions used in TPEFM readout.

In Vitro Culture During Retroviral Transduction Improves Thymic Repopulation and Output after TBI and Autologous PBPC Transplantation in Rhesus Macaques. R. Seggewiss[1], K. Loré[2], F. J. Guenaga[3], S. Pittaluga[4], R. E. Donahue[1], A. Krouse[1], M. E. Metzger[1], R. A. Koup[2], C. Reilly[5], D. C. Douek[3], and C. E. Dunbar[1]; [1]Hematology Branch, [2]Immunology Laboratory and [3]Human Immunology Section, Vaccine Research Center, NIAID. [4]Hematopathology Section, NCI, [5]Division of Biostatistics, University of Minnesota.

Life-threatening infections due to dysfunction of the T-cell compartment after peripheral blood progenitor cell (PBPC) transplantation remain a major problem in clinical stem cell transplantation. In rhesus macaques we compared the effects of three clinically relevant autologous transplantation graft compositions on immune reconstitution after total body irradiation (TBI). The animals received $CD34^+$ cell dose matched grafts of either peripheral blood mononuclear cells, purified $CD34^+$ PBPC or purified $CD34^+$ PBPC that had been expanded for 96 hours in vitro and retrovirally-transduced with a vector containing the neomycin resistance gene. We evaluated the reconstitution of T-cells, B-cells, NK-cells, monocytes and dendritic cells. The animals receiving selected-transduced $CD34^+$ cells had the fastest overall recovery of T-cell numbers post-transplant. The ratio of naive T-cells versus memory T-cells was consistently higher in this group. In addition, these animals showed the highest TREC levels, fewer proliferating Ki-67^+ T-cells and better preserved thymic architecture. Our data therefore suggest that selected-transduced $CD34^+$ cell grafts are able to repopulate the thymus more efficiently and promote a higher output of naïve T-cells. Our findings have implications for the design of gene therapy trials as well as for the use of ex vivo expanded hematopoietic stem cells in transplantation.

Novel Defects in the Heart Following Conditional Ablation of Mouse Myosin II-B. A. Singh, Q. Wei, K. Takeda, C. Liu, Z. Yu, and R. Adelstein; Laboratory of Molecular Cardiology.

Ubiquitous ablation of nonmuscle myosin heavy chain II-B (NMHC II-B) in mice resulted in defects in the heart and brain, leading to lethality between E14.5 and E18.5 during embryonic development. To further understand the physiological roles of NMHC II-B in adult mice, here we use loxP/Cre strategy to ablate NMHC II-B in the heart or in the brain specifically. Our purpose was two-fold: Firstly to see if the defects in the brain and heart were independent of each other and secondly, to avoid the early lethality of the ablated mice. Mice specifically ablated for NMHC II-B in the brain are smaller, display an absent or markedly reduced size of the subcommissural organ (SCO) and manifest abnormalities in the crossing of their posterior commissure axons. They develop a severe hydrocephalus most likely due to the abnormalities in the SCO and die between 12 and 22 days after birth, without showing any cardiac defects. In contrast, mice deficient for NMHC II-B only in the heart do not show any defects in the brain. However, most of these Bflox-/Bflox- mice display a novel phenotype beginning six months after birth. The first changes were manifested by EKG abnormalities, including bradycardia and defects in the conduction system including a posterior hemiblock. At nine months echocardiography showed a marked decrease in the fractional shortening (21% vs 47%) indicating that cardiac function was compromised. Pathological changes in the heart such as interstitial fibrosis, vacuolation, and infiltration of inflammatory cells were also seen at this time. Based on EM results, these mice also showed abnormalities of the Z-lines and structural changes in the intercalated discs, which is of note since NMHC II-B has been localized to both of these structures. Taken together, these findings suggest that NMHC II-B plays distinct physiological roles in the heart and brains of the newborn and adult brains and heart, respectively.

Computational Studies of Chaperonin-mediated Protein Folding. G. Stan[1], B. R. Brooks[1], D. Thirumalai[2], and G. H. Lorimer[2], Laboratory of Computational Biology, [1]National Heart, Lung and Blood Institute; [2]Institute for Physical Science and Technology and Department of Chemistry and Biochemistry, University of Maryland.

Protein folding mediated by chaperonin molecules is studied by computer simulations. Our focus is on the GroEL-GroES chaperonin complex of the Escherichia coli. The GroEL apical domain fragment (minichaperone) is able to assist folding of non-stringent substrate proteins through a mechanism of binding and unbinding. Molecular dynamics simulations of a peptide interacting with the apical domain show that the annealing efficiency is determined by the interplay between the interactions of the charged and hydrophobic residues in the helices H and I of the apical domain with the peptide. The fundamental requirement of the minichaperone annealing mechanism is a transient interaction of the substrate protein with the hydrophobic surfaces followed by its release by thermal fluctuations. We describe a sequence-based approach to identify the natural substrate proteins (SPs) for GroEL. Our method is based on the hypothesis that natural SPs are those that contain patterns of residues similar to those found in either GroES mobile loop and/or strongly binding peptide in complex with GroEL. The method is validated by comparing the predicted results with experimentally determined natural SPs for GroEL. We have searched for such patterns in five genomes. In the E. coli genome we identify 1422 (about a third) sequences that are putative natural SPs. A limited analysis of the predicted binding sequences shows that they do not adopt any preferred secondary structure. Our method also predicts the putative binding regions in the identified SPs. The results of our study show that a variety of SPs,associated with diverse functions, can interact with GroEL.

Increased Energy Dissipation in Cyclic Nucleotide Phosphodiesterase 3B (PDE3B) Knockout Mice. Y. Tang[1], O. Gavrilova[2], Y. Choi[1], S. Hockman[1], E. Billings[3], V. Manganiello[1]; [1]Pulmonary Critical Care Medicine Branch, [2]National Institute of Diabetes Digestive Kidney Diseases, [3]Bioinformatics Core Facility.

Although it is well-established that phosphodiesterase 3B(PDE3B) mediates the anti-lipolytic action of insulin in adipocytes, the role of PDE3B in lipid and energymetabolism remains to be determined. We show here that targeted inactivation of PDE3B (PDE3B KO) resulted in increased expression of genes required for fatty acid oxidation and energy dissipation in white adipose tissue (WAT), including UCP1, PGC-1a, PPARa, enzymes for fatty acid ß-oxidation, and macromolecules for mitochondrial biosynthesis. Interestingly, CIDE-A, which antagonizes the action of UCP1, was also induced in WAT in PDE3B KO mice. These results might explain why PDE3B KO mice exhibited phenotypic characteristics of lower body fat, increased oxygen consumption in response to ß3 adrenoreceptor agonist stimulation, and a smaller increase in body weight during high-fat diets, but without lipodystrophy. In parallel, inhibition of PDE3 in 3T3-L1 adipocytes with cilostamide potentiated the induction of UCP1 gene expression in the presence of ß3 adrenoreceptor agonist or cAMP analogue 8-CPT-cAMP. Furthermore, AMPKß1 subunits were increased in WAT from PDE3B KO mice. These results provide insight into the significant increases in fatty acid oxidation in isolated epididymal adipocytes from PDE3B KO mice. In addition, mRNA levels of macrophage-restricted proteins were decreased in epididymal adipose tissue from PDE3B KO mice. These results indicate that in PDE3B KO mice, white epididymal adipose tissue assumes some phenotypic characteristics of brown adipose tissue, and suggest that PDE3B might serve as an important regulator of lipid and energy metabolism.

Drosophila Myosin V: Solution Kinetics and Motile Properties. J. Toth, M. Kovacs, F. Wang, J. R. Sellers; Laboratory of Molecular Physiology.

Myosin V is the best characterized vesicle transporter myosin motor in vertebrates but it is unknown whether all members of the myosin V family share a common, evolutionarily conserved, mechanism. To address this question, we performed steady-state and transient kinetic measurements on a recombinant Drosophila myosin V S1 fragment. Interestingly, none of the product release steps can be identified as a single rate-limiting step. The ADP release rate constant from acto-S1, which is the bottleneck in the vertebrate myosin V ATPase cycle, appears to be 5-fold higher than the maximal steady-state ATPase activity. The Pi release rate constant from the acto-S1-products complex is even faster, implying that Drosophila myosin V does not follow a myosin II-type kinetic pattern, either. The key features of the ATPase cycle are the low ATP hydrolysis equilibrium constant (0.33) and the high steady-state actin attachment (96% at 5 M actin). The behavior of double-headed Drosophila myosin V in an in vitro motility assay suggests that a single molecule of myosin V cannot take multiple steps on the actin filament while being attached to it. We propose a mechanism whereby the ATP hydrolysis and the so-called weak actin-binding equilibria define the steady-state ATPase rate rather than the product release process. This mechanism suggests that Drosophila myosin V will not act as a single molecule vesicle transporter, in contrast to vertebrate myosin Vs. However, it may be able to perform processive transport if present in small ensembles on the surface of its cargo.

E3 ubiquitin ligase activity of the trifunctional ARD1 (ADP-ribosylation factor domain protein 1). A. Vichi, D. M. Payne, G. Pacheco-Rodriguez, J. Moss, and M. Vaughan; Pulmonary-Critical Care Medicine Branch.

Protein ubiquitinylation, which is involved in many important cellular processes, requires the E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and a substrate-specific E3 ubiquitin-protein ligase. The catalytic domain of one class of E3 ubiquitin ligases contains a zinc-binding RING finger motif. ARD1, with a RING finger domain near the N-terminus, two predicted B-Boxes, and a coiled-coil protein interaction motif immediately preceding an ARF domain at the C-terminus, belongs to the TRIM (Tripartite motif) or RBCC (RING, B-Box, coiled-coil) protein family. The B-Box and coiled-coil region serves as a GTPase-activating protein (GAP) for the ARF domain. We assessed E3 ligase activity of recombinant human ARD1 in vitro with mammalian E1, an E2 enzyme (UbcH6 or UbcH5a, 5b, or 5c), ATP, and ubiquitin. Multiple ubiquitinylated proteins, ranging in size from <90 to >220 kDa were separated by SDS-PAGE and characterized by immunoblotting. Deletion of the RING region or point mutations within the RING sequence abolished ARD1 E3 ligase activity. The RING finger domain itself (residues 1-110) was also active. All of our in vitro data are consistent with ARD1-catalyzed auto-ubiquitinylation. The substrate(s) for ARD1 E3 ubiquitin ligase activity and the relationship between E3 and ARF domain functions in cells, remain to be determined.

Sulfation Is Responsible for Altered Axonal Growth on Astrocytes. H. Wang, Y. Katagiri, H. M. Geller; Developmental Neurobiology Group.

The sulfation groups of chondroitin sulfate glycosaminoglycan (GAG) chains have important roles not only in adding negative charge to GAG chain, but in forming functional structure, axonal growth has been considered to be altered in response to different pattern of sulfation. Sulfation of GAG chain is accomplished by a family of specific enzymes called sulfotransferase. Here we found that the transcript of chondroitin-4-, but not chondroitin-6-sulfotransferase was up-regulated by cytokine TGF- β1 treatment with quantitative real time RT-PCR, and the increase in chondroitin-4-sulfotransferase protein was confirmed by immunoblotting data. Overexpression of C4ST in the mouse astrocytes and astrocytic cell line Neu7 displayed inhibitory effect to axonal growth when neuron plated on those astrocytes, while the effect of knocked down C4ST to axonal growth is under investigation. In vivo study in cortical injured mice, C4ST1, C6ST1 and C46ST transcripts were up-regulated at post lesion 3 days in ipsilateral side. Certain sulfotransferase mRNA level was also examined in different developmental stage by real time PCR. Taken together, our data indicated that sulfation pattern is responsible to altered axonal growth following TGF- β1 treatment as well as injury response.

A Comparison of Susceptible and Resistant Mice to Drug-Induced Liver Disease Using Genomics, Proteomics and SNPs Analyses. K. Welch, B. Wen, Y. Guo, M. Adams, T. Reilly, D. Goodlett, E. Yi, H. Lee, M. Radonovich, C. Pise-Masison, T. Hays, M. Bourdi, G. Peltz, J. Brady, S. Nelson, and L. Pohl; Laboratory of Molecular Immunology.

Drug-induced liver disease (DILD) causes significant morbidity and mortality and impairs new drug development. Recent evidence suggests that DILD is a complex, multi-factorial disease in which no one factor is likely to be responsible for susceptibility. In order to identify the multiple factors involved, we used more global approaches aimed at uncovering combinations of differences that might more aptly mimic situations seen in humans. Susceptibility differences to the well-known hepatotoxic drug, acetaminophen (APAP), were investigated using various strains of mice. Hepatic gene expression profiling following APAP administration revealed an elevated mRNA expression of numerous cytoprotective factors in resistant mice compared to the susceptible mice. Additionally, quantitative proteome analysis of livers from resistant and susceptible mice before and after treatment with APAP revealed that resistant mice have comparatively higher levels of several potentially important protective factors. There also appeared to be a loss of numerous mitochondrial proteins from the livers of susceptible mice after APAP treatment, suggesting that the loss of functional mitochondria may indeed play a role in APAP-induced liver injury. Additionally susceptibility to APAP-induced hepatotoxicity was correlated to known polymorphisms in 15 strains of mice. These findings suggest that comparative genomic and proteomic analyses of susceptible and resistant mouse strains may lead to the identification of multiple factors, which could contain critical polymorphism(s), and consequently have a role in determining susceptibility of individuals to DILD.

Improved Fluorophore-assisted Carbohydrate Electrophoresis (FACE) Analysis of Glycosaminoglycans. X. Wen, H. M. Geller, and Y. Katagiri; Developmental Neurobiology Group.

Proteoglycans consist of a core protein and an associated glycosaminoglycan (GAG) chain of chondroitin sulfate, heparan sulfate, dermatan sulfate, or keratan sulfate. Chondroitin sulfate proteoglycans (CSPGs), for example, are involved in neuronal development and injury of central nervous system, and we and others have demonstrated the importance of GAG chains in axon guidance. More specifically, sugar modifications such as sulfation are likely to act instructively in guidance decisions. Thus, analyses of the fine structure of GAGs are increasingly valuable for understanding many biological processes. Fluorophore-assisted carbohydrate electrophoresis (FACE) has been recently developed and has demonstrated a significant advantage in the microanalyses of GAGs compared to HPLC and NMR methods. Commercial products for FACE are available at a high cost and their resolution was insufficient. We have modified FACE procedure to obtain better results. Monosaccharides and unsaturated disaccharides with sulfation in various positions were fluorotagged by reductive amination with 2-aminoacridone, followed by electrophoresis in polyacrylamide gels prepared in the laboratory. We have tested various conditions to separate the labeled sugars and found that gels (37.5% polyacrylamide in Tris-Glycine buffer) gave us the best resolution with Tris-borate running buffer. Under this condition, disulfated disaccharides were completely separated from trisulfated disaccharide and the separation of monosaccharides was optimum. Thus, our modified FACE procedure represents a significantly improved approach for accurate compositional microanalyses of GAG chains.

Local Delivery of Bone Marrow Stromal Cells into Ischemic Muscle Augments Perfusion via Paracrine Mechanisms Rather than Endothelial Differentiation. A. Wragg, C. Graham, M. Konoplyannikov, M. Ma, S. Ding, and M. Boehm; Cardiovascular Branch.

Fractions of bone marrow stromal cells (MSCs) such as Multi-potent adult progenitor cells (MAPCs) have been reported to differentiate into cells of all three germ lineages. Injection of bone marrow derived cells, including MSCs, improves perfusion and function of ischemic tissues. Clinical studies are already underway. The optimal method for processing cells has not yet been established. We hypothesized that by culturing MSCs in MAPC specific media we could increase their differentiation potential and ability to improve perfusion and function of an ischemic muscle. Rat MSCs were cultured at low density in MAPC media and stably transduced with beta galactosidase to allow cell tracking in vivo. The cells' phenotype was consistent with MSCs by FACS. After endothelial, hepatocytic or neuronal differentiation, tissue specific antigens were up regulated as measured by immuno fluorescence (NSE, nestin and NF200 were induced after neuron, albumin and CK18 after hepatocyte and Dil LDL uptake and RECA1 staining after endothelial differentiation). A rat hind limb ischemia model was established. Ischemia was maintained for up to 28 days (perfusion 48% by laser Doppler imaging (LDI) and 62% by Fluospheres) and resulted in decreased treadmill running distance (pre-op 481m +/- 24, post ischemia 357m +/- 10 p=0.006). Syngeneic MSCs injected into ischemic limbs engrafted into the adventitia of arterioles in large numbers but did not adopt an endothelial phenotype. Limb perfusion increased after cell injection when measured by Fluospheres (adductor: cells 102% +/- 14, control 62% +/- 10 p= 0.03 gastrocnemius: cells 97% +/- 19, control 64% +/- 11 of normal p= 0.1). Interestingly superficial perfusion of the skin of the foot measured by LDI was not improved (cells 48% +/- 5.3 of normal, control 47% +/- 1.3 of normal) indicating that the skin and muscle vascular beds respond differently to cell therapy. Treadmill running was not improved by cell injection (cells 357m +/- 10, control 374m +/- 11). In summary: MSCs cultured under MAPC conditions can express tissue specific antigens from all 3 lineages after differentiation. MSCs improve muscle perfusion of ischemic hind limbs presumably by an augmentation of collateral remodeling by paracrine mechanisms rather than de novo vasculogenesis. MSCs do not improve treadmill running.

Curing of [PSI$^+$] Yeast Cells by Hsp104 Inactivation Does Not Require Cell Division. Y.-X. Wu[1], L. E. Greene[1], D. C. Masison[2], and E. Eisenberg[1]; [1]Laboratory of Cell Biology, [2]Laboratory of Biochemistry and Genetics, NIDDKD.

Hsp104 activity is required to maintain the yeast prion *[PSI$^+$]*, a self-propagating aggregated form of Sup35p. One model to explain this phenomenon proposes that, in the absence of Hsp104 activity, Sup35p aggregates enlarge but fail to replicate thus becoming diluted out as the yeast divide. To test this model we followed Sup35-GFP behavior after treatment of yeast with guanidine hydrochloride to inactivate Hsp104. About 30 minutes after guanidine hydrochloride addition there was a marked increase in formation of Sup35-GFP aggregates. Surprisingly, however, even before the yeast began to divide, these aggregates began to dissolve and after about 6 hours the Sup35-GFP looked identical to the Sup35-GFP in *[psi$^-$]* cells. Although plating studies showed that these yeast were still *[PSI$^+$]*, the observation that inactivation of Hsp104 decreased Sup35-GFP aggregation suggested that, over longer periods of time, inactivation of Hsp104 might cause yeast to become *[psi$^-$]* even if they were not dividing. We therefore added alpha mating factor to inhibit cell division. After 30 hours of guanidine hydrochloride incubation most of the cells exposed to alpha factor had not divided while control cells had divided about 15 times yet in both cases plating showed that 65% of the cells had become *[psi$^-$]*. After 40 hours both the control cells and the cells exposed to alpha factor were 95% *[psi$^-$]*. These results show that cell division is not a determining factor for curing the *[PSI$^+$]* phenotype when guanidine hydrochloride inactivates Hsp104 activity. Rather, curing apparently occurs because Sup35-GFP polymers slowly depolymerize independently of Hsp104 activity. Hsp104 then counteracts this curing possibly by catalyzing formation of new polymers.

Interaction of BIG2, a brefeldin A-inhibited guanine nucleotide-exchange protein, with exocyst protein Exo70. K. F. Xu, X. Shen, H. Li, G. Pacheco-Rodriguez, J. Moss, and M. Vaughan; Pulmonary-Critical Care Medicine Branch.

Guanine nucleotide-exchange proteins activate ADP-ribosylation factors by accelerating the replacement of bound GDP with GTP. Mammalian brefeldin A-inhibited guanine nucleotide-exchange proteins, BIG1 and BIG2, are important activators of ADP-ribosylation factors for vesicular trafficking. To identify proteins that interact with BIG2, we used cDNA constructs encoding BIG2 sequences in a yeast two-hybrid screen of a human heart library. Clone p2-5-3, encoding a form of human exocyst protein Exo70, interacted with BIG2 amino acids 1-643 and 1-832, but not 644-832, which was confirmed by coimmunoprecipitation of in vitro-translated BIG2 N-terminal segments and 2-5-3. By immunofluorescence microscopy, endogenous BIG2 and Exo70 in HepG2 cells were visualized at Golgi membranes and apparently at the microtubule-organizing center (MTOC). Both were identified in purified centrosomes. Immunoreactive Exo70 and BIG2 partially or completely overlapped with gamma-tubulin at the MTOC in cells inspected by confocal microscopy. In cells incubated with brefeldin A, most of the BIG2, Exo70, and trans-Golgi protein p230 were widely dispersed from their perinuclear concentrations, but small amounts always remained, apparently at the MTOC. After disruption of microtubules with nocodazole, BIG2 and Exo70 were widely distributed in cells and remained only partially colocalized with p230, BIG2 more so than Exo70. We conclude that in HepG2 cells BIG2 and Exo70 interact in trans-Golgi network and centrosomes, as well as in exocyst structures or complexes that move along microtubules to the plasma membrane, consistent with a functional association in both early and late stages of vesicular trafficking.

The Hepatoprotective Role of Interleukin-13 in Drug-induced Liver Disease. S. B. Yee, M. Bourdi, and L. R. Pohl; Laboratory of Molecular Immunology.

One predisposing aspect of idiosyncratic drug-induced liver disease (DILD) may involve a deficiency in hepatoprotective factors, such as interleukin (IL)-10, IL-6 and cyclooxygenase-2-derived mediators. In this regard, we tested the hypothesis that IL-13 is another hepatoprotective factor. Following treatment of male C57BL/6 wild-type (WT) mice with acetaminophen (APAP; 200 mg/kg), hepatotoxicity - as determined by serum alanine aminotransferase activity - peaked by 24 hours with a concomitant increase in serum IL-13 concentration. Administration of an IL-13 neutralizing antibody two hours before APAP treatment attenuated serum IL-13 concentration and significantly exacerbated liver injury up to 24 hours after APAP treatment. The protective role of IL-13 in APAP-induced liver injury was confirmed with the administration of APAP to IL-13 knockout (KO) mice. No difference, however, was observed in liver glutathione, APAP-adduct formation, or cytochrome P450 2E1 expression levels between WT and KO mice following APAP-treatment. This suggested the exacerbated liver injury in APAP-treated IL-13 deficient mice was not due to an enhancement in APAP bioactivation but rather secondary events in APAP-induced liver injury. Indeed, a number of protoxicant-related mediators become elevated in IL-13 KO mice after APAP-treatment, including serum tumor necrosis factor-a, interferon-γ and nitric oxide. Taken together, these results suggest that IL-13 is a critical hepatoprotective factor in APAP-induced liver injury, modulating the expression of protoxicant mediators. Further elucidation of the hepatoprotective role of IL-13 will be useful in better understanding the mechanism and in facilitating the prediction of DILD, thereby preventing the removal of otherwise beneficial drugs from the market.

ENU Induced Mutations Causing Congenital Cardiovascular Anomalies. Q. Yu, Y. Shen, B. Chatterjee, B. Siegfried, L. Leatherbury[1], C. Spurney, Y. J. Wu, C. W. Lo; Laboratory of Developmental Biology.

In our ongoing noninvasive fetal ultrasound screen of N-ethyl N-nitrosourea mutagenized mice, 11,554 mouse fetuses have been scanned, from which 471 fetuses derived from 192 families were identified as having cardiovascular defects. Represented were most of the major congenital cardiovascular anomalies seen clinically, including several families with phenotypes similar to human syndromes. The ENU induced mutations in two families were previously identified as Sema3CL605P and Gja1W45X - the former causing persistent truncus arteriosus (PTA) with interrupted aortic arch, and the latter conotruncal heart defects. The mutations in 6 other families have been mapped. Two mutations were mapped to mouse chromosome 4, one causing transposition of the great arteries and heart situs anomalies, and another with phenotypes reminiscent of Holt-Oram syndrome. Another two mutations were mapped to different regions of mouse chromosome 2, and both caused PTA, but one was additionally associated with craniofacial defects - phenotypes similar to DiGeorge syndrome. Another mutation that caused hypertrophic cardiomyopathy with semi-dominant inheritance was mapped to the X chromosome. One family exhibiting a constellation of anomalies, including pulmonary atresia/stenosis, omphalocele, polydactyly, exencephaly, cleft palate, and enopthalmus had a mutation that mapped to mouse chromosome 17. These phenotypes show overlap with the Pitx2 KO mouse and clinically with Reiger syndrome. However, Pitx2 is not situated on chromosome 17. A large-scale DNA sequencing effort is underway to identify the mutated genes in these new mouse models. These ENU induced mutations hold promise in yielding new insights into the genetic basis for human congenital heart disease.

Characterization of Tumor Necrosis Factor Receptor 1 (TNFR1) Vesicles in Human Plasma. J. Zhang, F. I. Hawari, B. Shamburek, B. Adamik, M. Kaler, F. N. Rouhani, and S. J. Levine; Pulmonary Critical Care Medicine Branch.

Extracellular TNF receptors function as TNF-binding proteins that modulate TNF activity. TNF is a pro-inflammatory cytokine that regulates host defense and apoptosis. Extracellular TNFR1 is generated by two mechanisms, proteolytic cleavage of soluble TNFR1 ectodomains and release of full-length TNFR1 in the membranes of exosome-like vesicles. The goal of this study was to characterize TNFR1 exosome-like vesicles in human plasma. Western blots of human plasma demonstrated a 48-kDa TNFR1, which is consistent with its release in the membranes of exosome-like vesicles. Approximately 30 nm, irregularly shaped vesicles that reacted with antibodies directed against the TNFR1 extracellular domain were visualized by immunoelectron microscopy. The 48-kDa TNFR1 segregated with the low density lipoprotein (LDL) fraction, which also contained ApoB-100. Endo H and PNGase F treatment of human vascular endothelial cell proteins reduced the size of cell-associated TNFR1 from 55-kDa to 48-kDa, whereas there was no effect on the 48-kDa TNFR1, which is consistent with the conclusion that it represents a full-length, deglycosylated protein. The 48-kDa TNFR1 sedimented to a peak density of 1.11 g/ml, which is consistent with an exosome-like vesicle. In contrast, Apo-B sedimented to a peak density of 1.03 g/ml, which is consistent with a LDL particle. There was no correlation between serum LDL levels and TNFR1 levels in 31 normal volunteers. These data suggest that TNFR1 and LDL reside in distinct particles. Thus, human plasma contains TNFR1 exosome-like vesicles, which may play an important role in innate immunity via their ability to bind TNF and regulate TNF-mediated inflammatory events.

Characterization of the product of hydroethidine and superoxide: Intracellular superoxide detection by HPLC and limitations of fluorescence. H. Zhao, H. M. Fales, E. A. Sokoloski, R. L. Levine, J. V. Vivar, and B. Kalyanaraman; Laboratory of Biochemistry.

Hydroethidine (HE) or dihydroethidium (DHE), a redox-sensitive probe, has been widely used to detect intracellular superoxide anion. It is a common assumption that the reaction between superoxide and HE results in the formation of a two-electron oxidized product, ethidium (E^+), which binds to DNA and leads to the enhancement of fluorescence (excitation, 500-530 nm; emission, 590-620 nm). However, the mechanism of oxidation of HE by the superoxide anion still remains unclear. In the present study, we show that superoxide generated in several enzymatic or chemical systems (e.g., xanthine/xanthine oxidase, endothelial nitric oxide synthase, or potassium superoxide) oxidizes HE to a fluorescent product (excitation, 480 nm; emission, 567 nm) that is totally different from E^+. HPLC measurements revealed that the HE/superoxide reaction product elutes differently from E^+. This new product exhibited an increase in fluorescence in the presence of DNA. Mass spectral data indicated that the molecular weight of the HE/superoxide reaction product is 330, while ethidium has a molecular weight of 314. Using mass spectral and NMR techniques, the chemical structure of this product was determined as 2-hydroxyethidium (2-OH-E^+). Using an authentic standard, we developed a new high performance liquid chromatography (HPLC) approach to detect and quantitate the reaction product of HE and superoxide formed in bovine aortic endothelial cells (BAEC) following treatment with menadione or antimycin A to induce intracellular reactive oxygen species (ROS). Concomitantly, we used the spin trap 5-tertbutoxycarbonyl-5-methyl-1-pyrroline N-oxide (BMPO) to detect and identify the structure of ROS formed. BMPO trapped the superoxide formed extracellularly that was detected as the BMPO-OH adduct, using the EPR technique. BMPO, being cell-permeable, inhibited the intracellular formation of 2-OH-E^+. However, the intracellular BMPO spin adduct was not detected. The definitive characterization of the reaction product of superoxide with HE described in this study forms the basis of an unambiguous assay for intracellular detection and quantitation of superoxide. Analysis of the fluorescence characteristics of ethidium (E^+) and 2-OH-E^+ strongly suggests that the currently available fluorescence methodology is not suitable for quantitating intracellular superoxide. We conclude that the HPLC/fluorescence assay using HE as a probe is more suitable for detecting intracellular superoxide.

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VOLUNTEER FOR THE NHLBI FELLOWS ADVISORY COMMITEE

HELP PLAN THE 2006 FELLOWS RETREAT

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